1959

Physiological Studies on Bloat

Clarence Leonard Moore

PHYSIOLOGICAL STUDIES ON BLOAT

BY

CLARENCE LEONARD MOORE

A thesis submitted
in partial fulfillment of the requirements for the
degree Doctor of Philosophy at South Dakota
State College of Agriculture
and Mechanic Arts

August, 1959

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Doctor of Philosophy, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

ACKNOWLEDGMENTS

The author gratefully acknowledges the technical assistance, extensive counsel, guidance and encouragement of Dr. A. E. Dracy, Professor of Dairy Husbandry. Appreciation is expressed to Dr. D. F. Breazeale, Head of the Dairy Department, for providing assistance and financial aid. The assistance from Mr. M. Ferdows, graduate assistant, and Mr. G. Clark, herdsman of the central farm, is most appreciative. Thanks to Dr. V. Wallace, Assistant Professor of Biochemistry, for preparation of the alfalfa juice concentrate. The author is indebted to the advisory committee--N. A. Hartwig, Professor of Entomology and Zoology; Dr. H. H. Voelker, Associate Professor of Dairy Husbandry; Dr. L. M. Bender, Extension Economist; and Dr. M. L. Shane, Associate Professor of English, who kindly read the manuscript and made several helpful suggestions, plus giving me guidance throughout my program.

The author is particularly indebted to Lila Moore, wife of the author, for many helpful suggestions in writing this manuscript and for typing the material. Acknowledgment is also made to eight month old Kevin Moore for his fine cooperation while my wife was typing this thesis.

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INTRODUCTION

Bloat is a complex and confusing syndrome involving an interaction of plant and animal factors. It is characterized by an excessive accumulation of gas in the rumen due to the inability of the animal to eructate. This gas arises largely from microbiological fermentation of food in the rumen. Many "toxic" principles of plants have been incriminated either by directly inhibiting rumen motility and eructation or indirectly by changing the physical characteristics of the rumen ingesta. Animals are known to vary greatly in susceptibility to bloat. Variations in anatomical structures, activity of such structures and microbiological activity all might be animal factors which are involved.

The fact that bloat is probably caused by a combination of plant and animal factors might explain its sporadic and unpredictable nature of occurrence. Bloat also varies in degree of severity from day to day. The above phenomena complicate the management of leguminous pastures.

The development of the livestock industry and the use of legumes in pasture programs have grown very rapidly during the past decade. Many surveys have been made to determine the economic loss involved in death and decreased production. However, the loss from not using improved legume pastures is unmeasurable, and probably is much higher.

Recent studies have been directed towards the biochemical constituents of legumes that might contribute to bloat. Attention has also been given to the eructating mechanisms and other physiological changes occurring during induced tympany. A minimum of research has been conducted on the physiological changes which occur during naturally-occurring acute bloat, i.e. animals grazing on alfalfa pastures.

<u>Objectives</u>

The objectives of the research reported in this thesis include: (1) To study physiological effects on sheep when drenched with legume extracts, (2) to devise a technique for measuring blood pressure in animals, (3) to study the change in heart rate, respiratory rate and blood pressure in animals during naturally occurring acute bloat, and (4) to study the methemoglobin and total hemoglobin values for normal and bloated cows.

REVIEW OF LITERATURE

Anatomy and Physiology in Relation to Bloat

The construction of the ruminant stomach is such that large quantities of highly fermentable material may be present at certain times. The stomach of the ox is a voluminous organ having a capacity of from 20 to 60 gallons, depending upon the size of the animal (38, 85). Four distinct compartments of the stomach are present and are known as the rumen, reticulum, omasum and abomasum (see Fig. 1).

The abomasum is located to the right of the rumen, resting upon the abdominal floor having a capacity similar to the omasum. It has an important digestive function because of its digestive secretions, but is not believed to be related to the bloat syndrome.

The size of the omasum is 7 to 8 percent of the total for the entire stomach. It is located in contact with the anterior portion of the visceral surface of the rumen, and to the right of the median line. The main function of the omasum is trituration of ingesta and is not believed to be related to bloat.

The reticulum is a comparatively small (5 percent of the stomach capacity) compartment located just anterior to the rumen cavity. Reticular motility and rumen motility, both concerned with the eructating mechanism, may be important physiological processes related to bloat.

In an adult animal, the rumen occupies 80 percent of the available space within the stomach. It lies in contact with the left abdominal wall and covers practically this entire surface. Because of this tremendous fermentation vat, bloat is characterized by an accumulation of gas within the rumen, and a distention of the left abdominal wall.

The ruminal gases formed are mainly from fermenting food ingested into the rumen. Rumen fermentation occurs and rumen gases are present early in an animal's life. Marshall et al. (65) found that rumen contents already exceed those of the abomasum between 7 and 30 days of age. According to Dukes (38), in the newborn calf the rumen and reticulum together are about one-half as large as the abomasum. At 10 to 12 weeks, the abomasum is about one-half as large as the rumen and reticulum combined. At four months of age, the rumen and reticulum together are about four times as large as the omasum and abomasum combined.

A majority of the ruminal gases are eliminated via eructation, and most of the remaining gases are probably diffused through the rumen wall. Eructation is a reflex (19, 82) which is dependent upon the reflex opening of the cardiac orifice (64). Eructation also involves reticuloruminal motility and the esophagus. Therefore, the modern concept of bloat is that it is due to a failure of the eructation mechanism.

The normal eructation reflex is essentially the movement of free gas from the dorsal sac of the rumen, forward and downward to the cardia. According to Weiss (93), this is accomplished by: (a) a forward wave of contraction of the ruminal musculature; (b) opening of the cardiac orifice which is brought about by contraction of the rumino-reticular fold; and (c) the clearing of the cardia of fluid ingesta is influenced by relaxation of the reticulum.

Dougherty and his co-workers have shown the presence of three esophageal sphincters (see Fig. 1). The two caudal sphincters are cardial

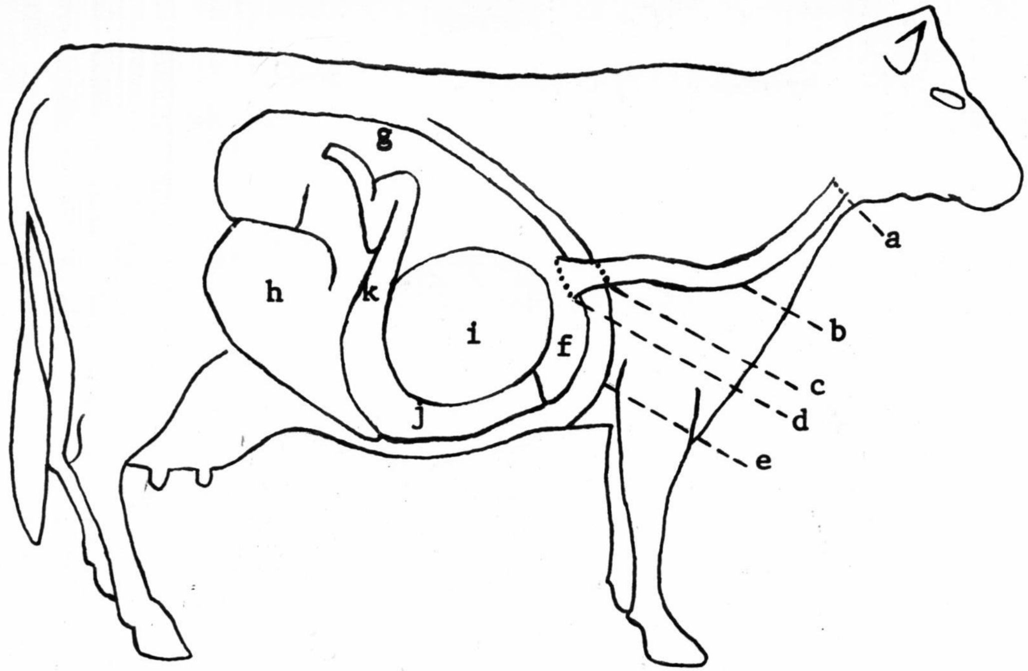

Figure 1. Anatomic Structures of the Polystomached Ruminant In situ:

a. Cranial esophageal sphincter
b. Esophagus
c. Prediaphragmatic esophageal sphincter
d. Cardia esophageal sphincter
e. Diaphragm
f. Reticulum
g. Dorsal sac of rumen
h. Ventral sac of rumen
i. Omasum
j. Abomasum
k. Duodenum

and diaphragmatic. The cardial sphincter is located at the cardia where the esophagus enters the stomach and the diaphragmatic sphincter is located farther cranial nearer the diaphragm. The third and more efficient sphincter is located at the cranial end of the esophagus (30, 31). According to Dougherty and Habel (30), eructation is composed of the following motor events:

(a) Two contractions of the reticulum.

(b) Contraction and raising of the ruminoreticular fold.

(c) General ruminal tonus or contraction forcing gas forward and downward into the relaxed and relatively empty reticulum.

(d) Increased eructation activity (contraction and dilation) of the esophagus.

(e) Relaxation of the two caudal esophageal sphincters.

(f) Gaseous distention of the esophagus.

(g) Continued closure of the cranial esophageal sphincter retaining the gas in the esophagus until the cardial and diaphragmatic sphincters close.

(h) Closing of the glottis causing a transient rise in intrapleural pressure which aids the esophageal musculature in rapidly clearing the esophagus of gas. This occurs through the relaxed cranial esophageal sphincter.

The main stimulus for eructation is gas pressure in the posterior dorsal sac of the rumen (93). Dougherty (25) and Nichols (71) have shown that increasing intraruminal pressure by insufflation increases ruminal activity and eructation. Wester (95) observed that eructation could occur in an animal with an open fistula and concluded that an increased intraruminal pressure is not essential to initiate the reflex. Other investigators believe that the stimulus for the eructation reflex is provided by roughage scratching on the rumen mucosa (18, 67). Schalk and Amadon (85) initiated the rumination reflex by scratching the mucosa of the reticulum. Dougherty and Meredith (31) have shown, through cinefluorographic methods, that an increase in gas pressure alone will greatly increase the rate of eructation.

Another factor that decreases the efficiency of the eructation reflex is the disturbances of the innervation of the stomach. The vagus nerve, which is parasympathetic, supplies excitatory fibers to the ruminant stomach as shown by Dougherty (25). When the vagus nerve is severed, the ability to eructate is lost and rumen motility ceases (35). Clark (7) concluded that the vagi are motor nerves to the rumen and reticulum.

Stevens and Sellers (90) have shown data which suggest that there are receptors for eructation which might be located in the rumen itself.

They concluded that the receptors for eructation are either stimulated directly by the contraction of certain rumen muscles or indirectly by the stimulation resulting from movement of ingesta during the rumen contraction. Dougherty <u>et al</u>. (33) reported the principal motor nerves supplying the esophagus are all branches of the vagus nerves.

Dziuk and Sellers (39) found that when the dorsal vagal trunk was stimulated with a moderate electrical stimuli there was an increased rate of rumen contraction and eructation. When a stronger electrical stimulus was applied, there were spasmodic contractions of the rumen, reticulum and cardia. Iggo (53) and Williams (96) indicate the receptors are located around the cardia area. Weiss (93, 94) postulates that the receptors are principally in the wall of the posterior dorsal blind sac.

Cinefluorographic studies on eructation have shown several phenomena relating to eructation. Apparently, eructation can be accomplished when the cardia is either partially or completely submerged in ingesta, but the cardia functions less efficiently (31). Dougherty and Habel (30) consider that there are receptors involved in the eructation reflex which can distinguish between gas pressure, which stimulates the cranial esophageal sphincter to open, and fluid pressure, which does not. This may explain why frothing of the rumen ingesta has been observed to influence the reflex act. Since the foam will not initiate the cranial sphincter to open, it cannot be belched out.

In the normal animal, eructation appears to be closely related to rumen and reticular motility. However, the esophagus does not have to open with every ruminal contraction (95) and likewise eructation can be accomplished during rumen paralysis (14, 31). It has been suggested (93) that an animal with a non-functioning reticulum causes the rumen to overfill more easily over the cardiac orifice thereby decreasing the efficiency of eructation. Dracy and Moore (36) removed approximately 80 percent of the reticulum in an animal, and approximately two months after the operation this animal died of bloat. This experiment by Dracy and Moore further suggests that reticulum motility may be vital to eructation.

The Cause of Death

The cause of death in bloat is not known. Much has been written, but little concrete information is known, about the final phase of acute fatal bloat. The two main theories are:

(1) Mechanical interference with respiration and blood flow.

(2) Toxic factors diffused from the rumen into the blood.

Theory No. 1.

It has been suggested (73) that as pressure due to gas in the rumen increases, pressure against the diaphragm becomes so great that it presses against the vital organs such as the heart, lungs and great veins. This not only prevents normal breathing, but also normal heart action which may eventually result in death. Another concept is that abdominal distention becomes so great in bloated animals as to cause complete immobilization of the diaphragm with its dome extending far forward into the thoracic cavity. The resultant asphyxia, combined with obstruction in the return of venous blood from the posterior vena cava, is responsible for the acute death (82). However, Dougherty, et al. (32) ligated the posterior vena cava of a dog and found it only had a transient and slight effect on carotid blood pressure.

Dougherty et al. insufflated the stomachs of sheep with different gases and found that an increased intraruminal pressure caused a sharp rise in arterial (carotid) and venous (jugular) blood pressures (32). In the same experiments, the cerebrospinal fluid pressure increased during insufflation and dropped when the intraruminal pressure was released. Dougherty and Meredith (31) through the use of angiocardiogram determined on one sheep whether raised intra-abdominal pressure or anterior displacement of the diaphragm affected the circulation. They found no gross alteration of circulation or distortion of the great vessels when a pressure of 40 mm. of mercury was applied intraruminally.

The gas within the rumen is extremely variable. Olson (74) found that animals having died from bloat had ruminal pressure ranging between 60 and 70 mm. of mercury. Animals that Olson insufflated with air had ruminal pressures up to 90-100 mm. of mercury with no ill effects except discomfort. Dougherty (27) quotes pressures recorded on three bloated cattle that had died to be between 72 and 75 mm. of mercury. Nichols (71) has insufflated sheep with pressures as high as 500 mm. of mercury before death occurred which is three to six times higher than pressures recorded in cattle. Dougherty et al. (32) when insufflating sheep found a marked difference between individual animals in their tolerance to the same gas insufflated at the same pressure.

Dougherty and his co-workers also found that the composition of the blood gases was rapidly and markedly affected by the intraruminal pressure. Oxygen utilization was decreased as was the pulmonary circulation time under the influence of insufflation. This work indicates that the physiological events are complex and that death in bloat does not result entirely from asphyxia or interference with circulation and respiration. As pointed out, this work covers some physiological effects of insufflation, not physiological effects occurring during bloat and therefore more similar information is needed on naturally occurring bloat. These effects pose a problem because of the difficulty of examining field bloat with adequate equipment.

Theory No. 2.

The toxic factor theory was developed by Dougherty and Cello (28, 29) when they found a toxic substance in the rumen ingesta of cattle and sheep. Greater amounts of toxins were found in animals suffering from bloat and acute indigestion than are found in normal animals. These substances reduced blood pressure when injected intravenously into dogs; inhibited rumen motility in sheep; stimulated respiration and stimulated motor activity in the lower gut of dogs, goats and sheep.

Clark (8, 9) and Dougherty (25, 26) reported the occurrence of histamine in the rumen ingesta of steers that had died of bloat. It has been well established that intravenous injection of histamine results in rumen paralysis. Dain _et al._ (22) identified histamine and tyramine as toxic constituents in the rumen ingesta of experimentally overfed sheep. Weiss (94) found that an intravenous injection of 2 mg. of histamine into a sheep completely inhibited all ruminal movements and eructation. Evans and Evans (41) demonstrated that the intestinal motility of the rabbit was impaired when an intestinal section was bathed in clover or alfalfa juice solution.

Ferguson (44) showed that lucerne (alfalfa) juice contained a substance which inhibited the activity of the rabbit ileum _in vitro_. Further work by Ferguson _et al._ (47) has shown this substance to be flavone tricin. When quercetin, a flavone which has about the same muscle inhibiting power as tricin, was fed to sheep, no bloat was produced (46). These workers concluded that flavones probably do not play a part in the production of bloat (45). At the Agricultural Research Center, Lindahl and co-workers failed to produce any bloat symptoms when a water suspension of 25 grams of quercetin was administered into sheep (63). Parsons _et al._ (78, 79) found that samples of rumen contents from cattle dying from acute bloat and extracts from forages causing bloat inhibited the motility of the isolated rabbit intestine. These data presented on toxic factors may partially support the toxic substance theory for bloat and indicate there may be a parallelism between the muscle inhibiting activity of certain plant extracts and the expected potential of the plants to produce bloat.

There are other possible toxic factors that could be classed under the toxic theory; however, they will be discussed in the following section. The actual cause of death resulting from toxins is still vague. These may affect circulation and respiration in the animal or inhibit other vital processes in the animal to initiate death.

The two main theories presented may both play some part as to the cause of death. They have been inadequately investigated, but give some interesting leads for work along these lines which might elucidate the cause of death.

<u>Chemical Factors in Relation to Rumen Paralysis</u>

A considerable amount of work has been done on the ways in which chemical factors might affect the eructation reflex or the paralysis or loss of tone in the rumino-reticular musculature. Also, chemical and physical factors may influence the rate of gas production in the rumen (15).

Cyanides have been suggested as one possible factor relating to bloat. Evans and Evans (41) incriminated cyanide as the causative agent in the production of bloat. Prussic acid has been shown to cause complete paralysis of ruminal movement (83). Clark and Quin (11) tested the effects of potassium cyanide on inhibition of eructation in sheep and concluded that their experiment afforded no evidence for incriminating toxic factors in plants as being associated with the etiology of acute bloat in ruminants. They found no inhibition of eructation even when the sheep showed paralysis of the rumen. Weiss (94) found that small doses of potassium cyanide inhibited rumen contraction in sheep while large doses caused complete inhibition of all motility and eructation. Parsons et al. (77) orally administered extracts of trefoil to lambs which produced death within 9 to 30 minutes with symptoms of cyanide poisoning. Dougherty (27) pointed out that bridsfoot trefoil, which has a history of causing very little or no bloat, has 19 times as much cyanide as ladino clover. Therefore, there is considerable doubt as to the importance cyanide plays in the pathogenesis of bloat.

Alkali, when injected intravenously or dosed into the rumen, caused ruminal paralysis, according to Clark and Lombard (10). Weiss (93) found the average efficiency of eructation of sheep had a remarkable inverse correlation to the average CO_2 combining power of the blood and obtained complete rumen paralysis at pH 7.8. Clark and Lombard (10) have shown that the administration of alkali into the rumen causes ruminal paresis if the pH exceeds approximately 7.5. Ruminal paresis was also caused by intravenous injection of alkali. There was no corresponding effect demonstrated following the dosing or injection of acid.

Hale and King (49) when studying urea toxicity found that bloat occurred when urea was administered orally in doses that were fatal or nearly fatal. They believed that the toxicity was due to the formation of ammonium carbonate. Urea is rapidly broken down to ammonia in the rumen; if it accumulates, it is assumed that a rise in ruminal pH results and consequent ruminal paresis. Clark et al. (14) found that urea toxicity in sheep caused atony of the rumen, muscular spasms and sudden death due to circulatory failure. Clark postulated that the sudden death from muscular spasms and circulatory failure may be due to the production of toxic intermediary products by the ruminal flora which utilize the ammonia. Normally the active flora will rapidly utilize the ammonia as a source of nitrogen and so prevent its accumulation. Clark found that after dosing the rumen with urea, the toxic symptoms were associated with the formation of ammonia, and that a high pH of the ruminal contents could be prevented or alleviated by the administration of acid (14). Johns (59) has found that high ammonia levels can be maintained in the rumen of a sheep without any signs of bloat or toxicity.

The enzyme cholinesterase is the chemical mediator of the parasympathetic nerves which is essential for proper body functions. Cholinesterase hydrolyzes acetylcholine and therefore limits the duration of acetylcholine. An excess of acetylcholine may cause muscular spasms (60). Quin and Van Der Wath (83) observed a paralysis of ruminal movement when acetylcholine in doses of ten milligrams or more was injected intravenously (dilution 1:1000), but the paralysis was of short duration (ten minutes).

The enzymatic hydrolysis of acetylcholine is strongly inhibited by the alkaloids eserine, prostigmine and atropine. Several workers (12, 34, 76, 94) have used atropine to produce ruminal paralysis. Weiss (93) found that even small doses of atropine (30 mg.) were insufficient for complete ruminal paralysis, and resulted in complete inhibition of the eructation reflex. On the other hand, Dougherty and Meredith (31) used an acetylcholine blocking agent (a compound oc. 1052-1575 Sharpe and Dohme) which produced complete cessation of rumen motility, but had no effect on the esophagus or cardia. Eructation occurred even when the rumen was partially filled with water. Several workers have isolated a substance from white clover which inhibits the action of cholinesterase (51, 87). Alder (1) compared levels of cholinesterase in blood of cattle on winter feeding in yards with animals grazing on bloat-producing pastures. He found there were slight depressions of cholinesterase activity in 7 out of 10 cattle following grazing, but these could not be correlated with bloat. Yarns (97) found that pseudocholinesterase activity increased from two to three-fold when blood was tested on an acutely bloated steer. Some bloated cows showed an increase in pseudocholinesterase while other bloated cows showed no increase and sometimes decreased over their normal levels. Also, sheep which were drenched with alfalfa juice had an increased pseudocholinesterase activity following drenching.

Dougherty (25) showed that adrenalin causes marked depression both in strength and speed of ruminal contractions of cattle. Weiss (93) found that an intravenous injection of 1 cc. of adrenalin hydrochloride (1:1000) into sheep produced varying degrees of depression and even total paralysis of rumino-reticular activity. Eructation was infrequent during the period of depressed ruminal motility. Weiss concluded that it would be logical to assume that the liberation of adrenalin during excitement would tend to inhibit the eructation reflex and so contribute to the occurrence of bloat in conjunction with other factors.

Saponins is another distinct biochemical substance that has been referred to as a contributing factor in acute bloat. McCandlish (66), Olson (75) and Quin (82) have postulated that saponin alters the surface tension of the ruminal contents and that it might contribute to "frothy bloat" by the entrapment of countless bubbles of gases from fermentation.

Lindahl _et al_. (63) have illustrated that alfalfa saponins have pronounced physiological as well as surface tension activity and both are instrumental in inducing experimental bloat symptoms. Experimental bloat symptoms were produced in sheep by oral and by intravenous administration of a composite mixture of saponins isolated from alfalfa. These workers have isolated six saponins from alfalfa and believe there are more. They believe it is unlikely that all of the saponins possess the same physiological activity or that the various fractions will always be found in the same ratio in growing legumes. Analytical work conducted at the Agricultural Research Center indicated that the total saponin content of alfalfa may vary from approximately 0.5 to 2 percent or more.

Lindahl _et al_. (63) presented data which give the following indications: (1) Alfalfa saponins can interfere with the eructation mechanism through their physiological actions alone. (2) Alfalfa saponins can contribute to the formation of froth, and thus interfere with eructation in an indirect manner. (3) The composite alfalfa saponins have pronounced pharmacological activity. (4) Saponins reduce activity of the reticulum, esophagus and intestines as well as the rumen. (5) Saponins may have a direct action on the respiratory centers by causing an increased rate and then irregular pattern. (6) Saponins affect cell counts, cardiac action and blood pressure through complex actions.

These workers state, "it appears that while alfalfa saponins may contribute to ruminant bloat, it cannot be concluded that saponins, _per se_, are the cause of naturally occurring bloat."

Review of Experimental Production of Bloat

The difficulty in producing bloat at will has hampered many studies.

as Weiss (93) stated, "Perhaps the greatest single factor responsible for the failure to elucidate the pathogenesis of bloat has been the difficulty in reproducing the natural condition." Several workers (4, 18, 54, 62, 77, 88, 89, 91) have produced bloat experimentally; however, no one has been able to produce fatal bloat regularly. The methods of producing bloat which will be discussed are: pasture bloat; "feed lot" bloat; and special procedures.

Cole et al. (18) showed that certain essential conditions are necessary before pasture bloat can be expected to occur. Weiss (93) summarized these conditions as follows:

(1) The lucerne should be in an appropriate stage of development, e.g. crisp and succulent to allow for rapid feeding.

(2) Animals should be deprived of all hay and straw and the lucerne itself should be free of grasses, weeds and old dry, tough stems.

(3) The lucerne should have been provided with sufficient water to induce rapid growth.

Even though these conditions are fulfilled, bloat may or may not occur. However, the chances of producing bloat are much greater if the above three conditions are met.

Soil fertility has been associated with the occurrence of bloat. Cooper (21) believes that where soils are deficient in available phosphorous, bloat may be a critical problem. Harris et al. (50) have indicated that bloat may be a serious problem wherever the soil is sufficiently fertile to produce a heavy growth of clover. On the other hand, Barrentine et al.

(2) in grazing experiments with steers on ladino clover, found that neither the moisture content nor lushness of the clover appeared to be a factor contributing to the incidence or severity of bloat.

Many research workers have been able to induce "feed lot" bloat by feeding special rations, although many of these rations have failed to reproduce bloating conditions in other sections of the country. Smith et al. (89) have produced frothy bloat in cows by feeding a ration of ground corn, soybean oil meal, and two to four pounds of leafy alfalfa hay. Frothy "feed lot" bloat has also been produced on a diet consisting of barley 61%, soybean oil meal 16%, NaCL 1%, and 22% alfalfa meal, together with four pounds of alfalfa hay (55). They found a definite increase in the incidence and severity of bloat when the animals were kept on the bloat-producing ration over a period of eleven weeks.

Several special procedures have been used for producing bloat and for further studying physiological aspects relating to the bloat problem. Numerous workers (77, 62, 45, 68, 69, 70) have been able to produce bloat by drenching with juice expressed from legumes. Moore (68) found that only slight bloat in sheep was produced with alfalfa juice, with a considerable amount of variation among animals as to susceptibility to bloat. Others found a higher degree of incidence and severity of bloat from alfalfa juice; however, the animals were grazing legumes prior to drenching whereas the animals drenched by Moore were on alfalfa hay prior to drenchings.

It was found by Boda et al. (5) that bloat could be produced in cattle fed dehydrated alfalfa by drenching with soluble protein (fresh eggwhite). The eggwhite resulted in the formation of a stable foam which interfered with eructation. Later work by Boda (6) demonstrated that the bloat-producing ability of alfalfa is greatly reduced after commercial dehydration.

Lindahl *et al*. (62, 63) have produced bloat in sheep by dosing with alfalfa saponins. The degree of bloat ranged from slight to severe with some animals not bloating. Several animals died and it was often a prolonged death, sometimes as long as four days after the saponin was administered. Similar results were reported by Moore (68) when sheep were drenched with a concentrated alfalfa juice.

Surgery has also been useful for producing bloat, and in studying various aspects of the problem. During 1883, Ellenberger (40) demonstrated that chronic bloat could be produced by sectioning both vagus nerves in the neck region. Weiss (93) found that when the right ventral branch was sectioned in a goat, distention and chronic bloat resulted. Sectioning the left dorsal branch diminished the strength of ruminal contractions and eructation efficiency for the first three weeks with subsequent partial recovery. Dracy and Jordan (35) did experiments on sectioning the vagus nerves on sheep that had been on legume pasture. Bloat was produced, although the animals did not die during a 24-hour period after sectioning.

The use of drugs for producing bloat has been covered previously in chemical factors in relation to rumen paralysis and cause of death. Cyanide, atropine, alkali, histamine and adrenalin all act by affecting rumen motility and eructation.

Another procedure in which much research work has been carried out is by artificially introducing gas into the rumen (23, 24, 71).

This has been used extensively for studying the physiological effects on the animal resulting from various gases and pressures.

Theories as to the Cause of Bloat

The excessive gas formation theory was once considered the only cause of bloat. Cole *et al*. (16, 17) and Quin (81) have done extensive work on gas formation. They have concluded there may be considerable difference in rate of gas formation from different feeds. However, as long as the eructation mechanism is working normally, animals have the ability to eliminate far more gas through eructation than is produced in the rumen (19).

Jacobson *et al*. (57) supported the excessive consumption of dense feed theory. They suggested that bloat on green legumes occurs because legumes are eaten so rapidly that the feed "piles up" in such a manner that the cardiac opening is blocked, thus preventing eructation. Dougherty (32) has shown that eructation can be accomplished when the cardiac is either partially or completely submerged in ingesta. Nichols (71) and Weiss (93) reported similar results. Cole *et al*. (19, 20) indicated that it is the greedy eater that bloats due to the rapid ingestion of too much succulent forage. Johns (58) found no relationship between rate of food intake and severity of bloat. Many others, however, believe (36, 43, 68, 92) that rapid grazing, particularly during wet seasons when the forage is premature, results in many cases of bloat. On the other hand, bloat has often occurred during drought.

Nichols' mechanical or buoyancy theory (72) is a different approach to the part played by the density of the food. Accordingly, heavy feeds like immature alfalfa or ladino clover settle to the bottom of the rumen in a compact mass. The fermentation liberates many tiny gas bubbles which are trapped and tend to hold the liquid level above the opening of the lower end of the esophagus thus preventing the escape of gas. Johns' observations (60) do not confirm Nichols' views. When animals bloated on red or white clover, the ventral portion of the rumen content was almost completely liquid. The main collection of small bubbles was in the dorsal and middle regions and in the posterior ventral sac of the rumen.

The physical deficiency theory, advanced by Cole and associates (17) is based on the idea that scabrous material is required to stimulate eructations mechanically. Clark and Weiss (12) suggested that the role of the roughage is to stimulate salivary flow, ensuring a watery non-viscous rumen ingesta. They considered that bloating is due to foam formation in a viscid medium which is insufficiently diluted with saliva. Feeding roughage before allowing animals to graze a bloat-provoking pasture has been a widely accepted bloat preventive measure and supports this theory. Other investigators pointed out that bloat occurs even when supplemental feeding of scabrous roughage is practiced (64, 84). Dougherty (27) suggested that any major changes in the ration not only changes physical factors in the ingesta, but may also change the microflora and microfauna and thus have an important bearing on the chemical composition of the ingesta. Later experiments by Hungate et al. (52) verified this concept.

The surface tension theory as stated by Dougherty (27) embraces any material which will change surface tension so that the gases from fermentation will tend to accumulate in countless bubbles throughout the ingesta instead of rising to the top and collecting in a gas pocket above the ingesta. This type of bloat is referred to as frothy bloat. Frothy bloat is receiving considerable attention throughout the country, but how important it is in relation to the free gas type is a debatable question. Lindahl and Davis (61) found in "feed lot" bloat that the cause of bloat appeared to be from the formation of very stable frothy ruminal contents, although there was always some free gas in the rumen. Moore also observed that in bloat from alfalfa pasture there is always a pocket of free gas and then a frothy mass of ruminal contents. Removing just the free gas pocket generally was not enough to relieve a seriously bloated animal. Therefore, probably the frothing condition caused the animals to bloat. As Johns (60) stated, "The degree of bloat appears to be associated with the stability of the foam." This is supported by the work of Lindahl and Davis (61), Jacobson and Lindahl (54, 55), Jacobson *et al*. (56), and Wallace (92).

Two factors which are believed to alter surface tension are saponins and saliva. Quin (93) postulated that saponin increases the surface tension of the rumen contents and contributes to the development of frothy bloat. Clark and Weiss (13) showed that saliva may be another factor affecting surface tension. Weiss (93) reported that the formation of froth is dependent on the consistency of the ruminal ingesta which in turn is influenced by reflex salivary secretion, and the greater the salivary secretion, the less the tendency for froth formation. The salivary secretion is stimulated by the presence of coarse material in the ingesta.

The toxic gas theory or toxic factors diffusing from the rumen into the blood has been previously discussed; however, it is believed that these gases or factors, singly or in combination, partially paralyze the rumen thus preventing eructation. When the animal cannot eructate, the pressure increases and the toxic gases are absorbed into the blood. Dracy *et al.* (37) clamped the esophagus of sheep after they had grazed on an alfalfa pasture in order to determine whether or not death was due to the accumulation of gas within the rumen. The results were that the sheep bloated, but did not produce enough gas to cause death. This possibly indicates that a toxic factor is not present in alfalfa at all times.

According to Olson (74, 75) the percentage of gases, except hydrogen sulfide and methane, remain about the same in normal and bloated animals. It has been shown by Olson (74, 75) that both carbon monoxide and hydrogen sulfide are present in the rumen of normal animals, but that hydrogen sulfide is greatly increased in bloated animals. The assumption is that hydrogen sulfide, a highly toxic gas, paralyzes the rumen wall and is subsequently absorbed into the blood stream.

EXPERIMENTAL METHODS

The research presented herein consists of three experiments: (1) alfalfa extract, (2) carotid arterial blood pressure, and (3) methemoglobin and total hemoglobin.

Alfalfa Extract

Concentrated alfalfa juice was prepared and tested for its ability to produce bloat in lambs.

The alfalfa juice was prepared to concentrate the saponins or other toxic constituents by placing freshly cut alfalfa in a steam bath. This steam bath heated the material to coagulate the protein and also to facilitate the rate of grinding. The next step was grinding the material in a meat grinder. Following this, it was again steamed, which aided in removing the solid material. The liquid portion was separated from the solid portion by pressing out the juice with a lard press. The liquid portion was further concentrated by evaporating it in a steam jacket with forced air. The material was then refrigerated at 44° F until used for drenching lambs.

Thirteen Western lambs weighing approximately 80 pounds each were used in this experiment. Their ration consisted of alfalfa hay (ad lib.) and free access to salt and water whenever they were not being drenched.

The alfalfa juice extract was administered to the lambs via stomach tube. This tube was placed into the lambs' esophagus and then extended into the rumen. With a funnel in the anterior end of the stomach tube, a measured quantity of the juice was given to each lamb.

The quantity of juice used was calculated to equal approximately four and one-half percent of the lamb's body weight. For a lamb weighing

80 pounds, this calculates out to be approximately 1700 ml. The lambs were drenched with alfalfa juice having concentrations of 20, 15, 10, 5 and 0 percent solids. Therefore, the lambs drenched with 20 percent solids in the alfalfa juice concentrate received 1600 ml. of concentrate and 100 ml. of water. Water was needed to dilute the concentrate to facilitate passage down the stomach tube. The lambs drenched with 15 percent solids received 1200 ml. of concentrate and 500 ml. of water. Those drenched with 10 percent solids had 800 ml. of concentrate and 900 ml. of water. The lambs getting 5 percent solids received 400 ml. of concentrate and 1300 ml. of water. The control lambs received no concentrate and 1700 ml. of water which contained a one-percent glucose solution.

Recordings were made on heart and respiratory rates, abdominal circumference, and other resulting physical effects. The heart and respiratory rates and abdominal circumference were taken on each animal before and after each drench. These lambs were handled with as much ease as possible when taking these recordings. The recordings were made after the drench as soon as the lambs seemed to be quiet, which took approximately 15 minutes. Then the recordings were taken at hourly intervals for three hours after each drench.

The heart rate was taken by counting the beats per minute with the aid of a stethoscope and stop-watch. The stethoscope was also used in taking the respiratory rate by holding it over the area of the trachea and recording the inhalations per minute. The abdominal circumference was measured by placing a tape measure around the greatest circumference of the abdomen.

Carotid Arterial Blood Pressure

In the bovine, there has been no way of determining the blood pressure without creating abnormal conditions. Blood pressure has been determined by anesthetizing the animal and cannulating the carotid artery. This method has been used in insufflation experiments whereby the blood pressure was obtained on experimentally bloated animals. However, this technique could not be used on normally bloated animals because it is necessary to take the blood pressure as quickly as possible and with little disturbance to the animal.

Since there are no arteries of sufficient size in a usable position in the bovine for determining blood pressure, a technique was devised to exteriorize the carotid artery. This technique is similar to that used by Graham, et al. (48) and Schambey, et al. (86) for obtaining arterial blood samples. The carotid artery was brought to the outside and sutured into a flap of skin. The cuff of a sphygmomanometer could then be wrapped around the carotid artery in the skin flap (carotid loop) and the blood pressure obtained either by the palpatation or the auscultation method as done with human beings.

The carotid loop operation was performed as follows: The animal was fasted for 24 hours preceding the operation, and then anesthetized by injecting nembutal into the blood stream via the jugular vein. The amount of nembutal used was approximately 30 milligrams per kilogram of body weight. The ventral surface of the neck was clipped, scrubbed with soap and water, and washed with a 70% alcohol solution. Approximately an eight inch mid-line incision was made on the ventral surface of the neck. A second parallel incision was made about three inches from the first. The skin was then separated from the connective tissue between the two incisions. This made up the skin flap which was later wrapped around the carotid artery. The sternocephalicus and the omo-hyoideus muscles were separated. The artery and vagus nerves which were embedded in a sheath were brought to the surface and separated. The artery was placed in the skin flap and the flap was sewed shut. The muscles were sutured below and the two remaining edges of the incisions beneath the carotid loop were sutured together. This completed the operation. Much care was needed in suturing and good postoperative care of the animal was necessary until healing was complete.

Three Holstein-Friesian calves, two males and one female, were used in this experiment. They were approximately three months of age when the carotid loop operation was performed, and they were kept until 5-8 months of age when they were started on alfalfa pasture in June, 1958.

Systolic blood pressure, heart and respiratory rates were taken on these three calves with carotid loops. The diastolic pressure could not be determined whenever the animals had labored breathing. Recordings were made before the animals were pastured and after pasturing, or whenever they bloated. The animals were kept in dry-lot overnight to increase their hunger and consequent chance to bloat and had access only to salt and water.

Methemoglobin and Total Hemoglobin

Eighteen cows in the South Dakota State College dairy research herd were used in this study. Each day they were placed on an alfalfa pasture which should have bloat-producing potentials because of purity of alfalfa, irrigation, fertilization and stage of maturity. Whenever there was a case of bloat, methemoglobin and total hemoglobin were run on the bloated animals, normal animals and then on the animals that had bloated after they returned to normal.

Methemoglobin and total hemoglobin analyses were run according to the method described by Evelyn and Malloy (42). Blood samples were drawn in a heparinized tube and placed in a refrigerated box until analyzed. This analysis was run approximately 15 to 30 minutes after the blood sample was taken.

RESULTS

The results of these experiments are divided into three sections: (1) alfalfa extract, (2) carotid arterial blood pressure, and (3) methemoglobin and total hemoglobin.

Alfalfa Extract

Twenty percent solids - ten drenchings in nine lambs. Three animals died, and bloat was produced in nine out of ten drenchings. As shown in Table I, there was only one case of severe bloat (lamb no. 65). Lamb numbers 61 and 63 (which also died) bloated only slightly. This observation was made several hours before death and, therefore, the lambs could have bloated immediately before they died.

Heart rate increased immediately after drenching, but gradually returned to normal. Respiratory rate decreased from 118 inhalations per minute to 81 within the first hour after drenching and then increased to 84 and 103 the next two to three hours. The greatest abdominal circumference increase was five inches right after the drenching. These are the average values for all the lambs as indicated in Table II.

Fifteen percent solids - ten drenchings of eight lambs produced four cases of bloat. Three of the animals died several hours after the drenchings. Only one of the lambs that died showed signs of bloat, and this animal bloated five hours after drenching (Table III). This lamb's heart rate increased immediately after drenching and then dropped slightly below normal. Its respiratory rate decreased after the drenching and then gradually increased. The average abdominal circumference increase was four inches following drenching. All these facts can be depicted in Table IV.

TABLE I. RESULTS FROM DRENCHING LAMBS WITH ALFALFA JUICE CONCENTRATE (20 PERCENT SOLIDS)

Date Drenched	Lamb No.	Treatment: Concentrate (ml)	Treatment: Water (ml)	Remarks
7-18-57	61	1600	100	Slight bloat; died approximately 6 hrs. after drenching.
7-19-57	67	1600	100	Slight bloat
7-28-57	63	1600	100	Slight bloat; died during the night, approximately 15 - 20 hrs. after drenching.
7-29-57	65	1600	100	Severe bloat; died during the night, approximately 15 - 20 hrs. after drenching,
7-30-57	55	1600	100	Slight bloat
7-30-57	58	1600	100	Slight bloat
7-31-57	66	1600	100	Slight bloat
8-1-57	60	1600	100	Slight bloat
8-19-57	60	1600	100	No bloat
8-19-57	64	1600	100	Slight bloat

TABLE II. HEART RATES, RESPIRATORY RATES AND ABDOMINAL CIRCUMFERENCE WHEN ALFALFA JUICE CONCENTRATE (20 PERCENT SOLIDS) WAS ADMINISTERED IN LAMBS

		Before Drench			Within 1 Hr. After Drench			Between 1 & 2 Hrs. After Drench			Between 2 & 3 Hrs. After Drench		
Date	Lamb No.	HR	RR	AC	HR	RR	AC	HR	RR	AC	HR	RR	AC
7-18-57	61	120	72	43.5	144	120	50.0	126	96	49.0	126	96	48.5
7-19-57	67	114	78	37.5	168	66	43.0	138	84	42.0	132	90	42.0
7-28-57	63	150	54	36.0	180	54	41.0	132	54	39.5	132	60	39.0
7-29-57	65	168	162	39.0	180	54	43.0	132	96	41.0	138	108	41.5
7-30-57	55	96	204	36.5	102	102	41.5	108	60	40.0	108	144	40.5
7-30-57	58	108	192	36.0	114	54	41.0	102	84	41.5	102	96	40.0
7-31-57	66	108	132	37.5	168	54	42.5	174	108	41.0	180	120	41.0
8-1-57	60	138	168	38.5	108	114	45.0	138	66	43.0	150	136	43.5
8-19-57	60	144	48	43.5	132	84	45.5	126	42	45.0	150	54	45.0
8-19-57	64	132	66	38.0	156	108	42.5	168	150	42.0	144	126	41.0
Average		128	118	39.0	145	81	44.0	134	84	42.0	136	103	42.0
Change					+17	-37	+5	+6	-34	+3	+8	-15	+3

HR = Heart rate (min.)
RR = Respiratory rate (min.)
AC = Abdominal Circumference (inches)

TABLE III. RESULTS FROM DRENCHING LAMBS WITH ALFALFA JUICE CONCENTRATE (15 PERCENT SOLIDS)

Date Drenched	Lamb No.	Treatment Concentrate (ml)	Treatment Water (ml)	Remarks
7-18-57	59	1200	500	No sign of bloat; however, died during the night, approximately 15 - 20 hrs. after drenching.
7-19-57	58	1200	500	No sign of bloat
7-28-57	58	1200	500	No sign of bloat
7-29-57	67	1200	500	No sign of bloat; but died the next day, approximately 30 hrs. after drenching.
7-30-57	68	1200	500	No sign of bloat
7-30-57	60	1200	500	Slight bloat
7-31-57	62	1200	500	Slight bloat
8-1-57	54	1200	500	Moderate bloat 5 hrs. after drenching and died during the night.
8-1-57	64	1200	500	Slight bloat
8-19-57	58	1200	500	No sign of bloat

TABLE IV. HEART RATES, RESPIRATORY RATES AND ABDOMINAL CIRCUMFERENCE WHEN ALFALFA JUICE CONCENTRATE (15 PERCENT SOLIDS) WAS ADMINISTERED IN LAMBS

		Before Drench			Within 1 Hr. After Drench			Between 1 & 2 Hrs. After Drench			Between 2 & 3 Hrs. After Drench		
Date	Lamb No.	HR	RR	AC	HR	RR	AC	HR	RR	AC	HR	RR	AC
7-18-57	59	192	66	40.0	228	102	42.5	168	90	42.0	132	96	42.0
7-19-57	58	150	60	40.0	204	60	42.5	132	60	42.0	108	66	41.5
7-28-57	58	120	48	38.0	174	54	43.0	90	54	43.0	90	60	42.5
7-29-57	67	138	168	34.0	156	60	37.0	162	156	38.5	132	102	37.0
7-30-57	68	96	168	38.0	102	120	39.5	90	96	40.5	108	144	39.0
7-30-57	60	108	132	38.5	120	60	44.0	114	90	45.0	132	120	44.0
7-31-57	62	132	84	40.0	168	66	44.0	84	48	44.0	144	114	42.0
8-1-57	54	102	78	34.5	102	30	37.5	96	48	36.0	102	114	37.5
8-1-57	64	126	204	36.5	150	60	41.0	142	42	38.0	156	156	40.0
8-19-57	58	120	108	43.5	114	72	46.5	120	66	44.5	120	90	44.5
Average		128	112	38.0	152	68	42.0	120	75	41.0	122	106	41.0
Change					+24	-44	+4	-8	-37	+3	-6	-6	+3

<u>Ten percent solids</u> - four cases of bloat were produced in seven lambs on eleven drenchings. None of the animals died. No serious distress symptoms were noted even on the moderately bloated animals (Table V).

Table VI shows the heart rate, respiratory rate and abdominal circumference values. All followed the same general pattern as mentioned previously.

<u>Five percent solids</u> - nine drenchings on six lambs produced four cases of slight bloat and death in another animal (Table VII). Lamb no. 68 which died showed no signs of bloat while under observation. The physical symptoms were the same as all other lambs which died. They had no appetite, were very comatose, lay down most of the time and had somewhat labored breathing.

The heart rates, respiratory rates and abdominal circumference, as shown in Table VIII, followed the same general pattern as with the other drenches except the heart rate did not increase as much.

<u>Control, one percent glucose solution</u> - from nine drenchings, no bloat was produced (Table IX). This drenching was made each time the alfalfa extract was administered.

Table X shows no increase in heart rate after the drenching. The respiratory rate decreased as before, and the abdominal circumference increased only two inches after the drenchings.

<u>Summary of heart rates, respiratory rates and abdominal circumference from drenchings</u>. The comparison of the average heart rate for each type of drenching is shown on Figure 2. The heart rate increased right after the drenching for all alfalfa extracts, but not for the control with glucose. The average respiratory rate from each type of drench decreased

TABLE V. RESULTS FROM DRENCHING LAMBS WITH ALFALFA JUICE CONCENTRATE (10 PERCENT SOLIDS)

Date Drenched	Lamb No.	Treatment Concentrate (ml)	Water (ml)	Remarks
7-18-57	60	800	900	No bloat
7-19-57	62	800	900	No bloat
7-28-57	60	800	900	No bloat
7-29-57	58	800	900	Slight bloat
7-30-57	64	800	900	Moderate bloat
7-30-57	54	800	900	No bloat
7-31-57	64	800	900	Moderate bloat
7-31-57	54	800	900	No bloat
8-1-57	55	800	900	No bloat
8-1-57	66	800	900	Slight bloat
8-19-57	66	800	900	No bloat

TABLE VI. HEART RATES, RESPIRATORY RATES AND ABDOMINAL CIRCUMFERENCE WHEN ALFALFA JUICE CONCENTRATE (10 PERCENT SOLIDS) WAS ADMINISTERED IN LAMBS

		Before Drench			Within 1 Hr. After Drench			Between 1 & 2 Hrs. After Drench			Between 2 & 3 Hrs. After Drench		
Date	Lamb No.	HR	RR	AC	HR	RR	AC	HR	RR	AC	HR	RR	AC
7-18-57	60	126	102	41.0	144	138	46.5	132	120	46.0	126	120	45.5
7-19-57	62	126	144	34.5	144	54	38.5	132	98	40.5	126	98	40.0
7-28-57	60	150	42	38.5	108	54	44.0	108	54	42.0	108	60	42.0
7-29-57	58	108	180	39.5	120	138	43.0	138	72	42.5	156	168	42.5
7-30-57	64	120	216	36.5	168	48	40.5	120	162	42.0	144	204	41.0
7-30-57	54	120	108	35.0	114	48	40.0	114	66	40.0	102	144	39.0
7-31-57	64	108	174	35.0	162	108	40.5	114	102	39.0	144	120	38.5
7-31-57	54	102	48	33.0	120	36	36.5	120	66	37.0	108	78	37.0
8-1-57	55	102	90	36.0	90	30	38.0	102	42	37.0	96	132	37.0
8-1-57	66	108	108	37.0	120	54	38.5	132	60	43.0	126	60	42.0
8-19-57	66	108	54	39.0	120	66	43.0	126	60	41.5	120	96	42.0
Average		116	115	37.0	128	70	41.0	122	82	41.0	123	116	41.0
Change					+12	-45	+4	+6	-33	+4	+7	+1	+4

TABLE VII. RESULTS FROM DRENCHING LAMBS WITH ALFALFA JUICE CONCENTRATE (5 PERCENT SOLIDS)

Date Drenched	Lamb No.	Treatment Concentrate (ml)	Treatment Water (ml)	Remarks
7-18-57	68	400	1300	No bloat
7-19-57	64	400	1300	No bloat
7-28-57	62	400	1300	Slight bloat
7-29-57	62	400	1300	Slight bloat
7-30-57	62	400	1300	Slight bloat
7-31-57	68	400	1300	No bloat; but died during the night, approximately 15 - 20 hrs. after drenching.
7-31-57	60	400	1300	Slight bloat
8-1-57	58	400	1300	No bloat
8-19-57	55	400	1300	No bloat

TABLE VIII. HEART RATES, RESPIRATORY RATES AND ABDOMINAL CIRCUMFERENCE WHEN ALFALFA JUICE CONCENTRATE (5 PERCENT SOLIDS) WAS ADMINISTERED IN LAMBS

		Before Drench			Within 1 Hr. After Drench			Between 1 & 2 Hrs. After Drench			Between 2 & 3 Hrs. After Drench		
Date	Lamb No.	HR	RR	AC	HR	RR	AC	HR	RR	AC	HR	RR	AC
7-18-57	68	126	120	40.5	144	144	47.0	126	60	46.5	120	120	46.0
7-19-57	64	132	126	34.5	162	48	38.5	144	144	38.5	132	138	38.0
7-28-57	62	144	78	38.5	126	54	42.0	120	76	43.0	120	96	42.5
7-29-57	62	126	120	38.5	138	90	43.0	120	114	40.0	138	102	38.5
7-30-57	62	102	174	39.5	132	84	44.0	120	54	43.5	120	138	41.5
7-31-57	68	150	78	38.0	150	36	40.0	150	60	42.0	144	42	41.0
7-31-57	60	132	138	41.0	132	72	45.0	126	30	43.0	138	144	44.0
8-1-57	58	96	198	40.0	108	30	42.0	108	102	41.0	108	150	38.5
8-19-57	55	138	84	39.0	132	114	42.0	120	132	40.5	114	132	39.5
Average		127	124	39.0	136	75	43.0	126	86	42.0	126	118	41.0
Change					+9	-49	+4	-1	-38	+3	+2	-6	+2

TABLE IX. RESULTS FROM DRENCHING LAMBS WITH A ONE PERCENT GLUCOSE SOLUTION

Date Drenched	Lamb No.	Treatment Glucose (gm)	Treatment Water (ml)	Remarks
7-18-57	63	17	1700	No bloat
7-19-57	65	17	1700	No bloat
7-28-57	67	17	1700	No bloat
7-29-57	60	17	1700	No bloat
7-30-57	66	17	1700	No bloat
7-31-57	55	17	1700	No bloat
7-31-57	58	17	1700	No bloat
8-1-57	62	17	1700	No bloat
8-19-57	62	17	1700	No bloat

TABLE X. HEART RATES, RESPIRATORY RATES AND ABDOMINAL CIRCUMFERENCE WHEN ONE PERCENT GLUCOSE SOLUTION WAS ADMINISTERED IN LAMBS

		Before Drench			Within 1 Hr. After Drench			Between 1 & 2 Hrs. After Drench			Between 2 & 3 Hrs. After Drench		
Date	Lamb No.	HR	RR	AC	HR	RR	AC	HR	RR	AC	HR	RR	AC
7-18-57	63	144	66	40.0	144	102	44.0	144	84	42.5	126	96	42.0
7-19-57	65	144	48	36.5	126	60	37.0	114	84	37.0	114	108	36.5
7-28-57	67	150	54	38.0	138	30	38.5	138	84	38.0	120	102	37.0
7-29-57	60	138	168	41.0	132	114	42.5	102	108	41.0	120	144	40.5
7-30-57	66	108	162	38.0	102	66	38.5	96	84	39.0	102	144	38.5
7-31-57	55	120	36	38.0	120	36	39.5	108	48	39.0	102	60	37.0
7-31-57	58	96	144	38.5	96	42	41.0	102	30	40.5	108	78	40.0
8-1-57	62	96	156	38.5	114	42	40.5	114	132	38.0	108	108	38.0
8-19-57	62	120	72	42.5	114	90	44.0	108	96	43.0	108	66	43.0
Average		124	101	39.0	121	65	41.0	114	83	40.0	112	101	39.0
Change					-3	-36	+2	-10	-18	+1	-12	0	0

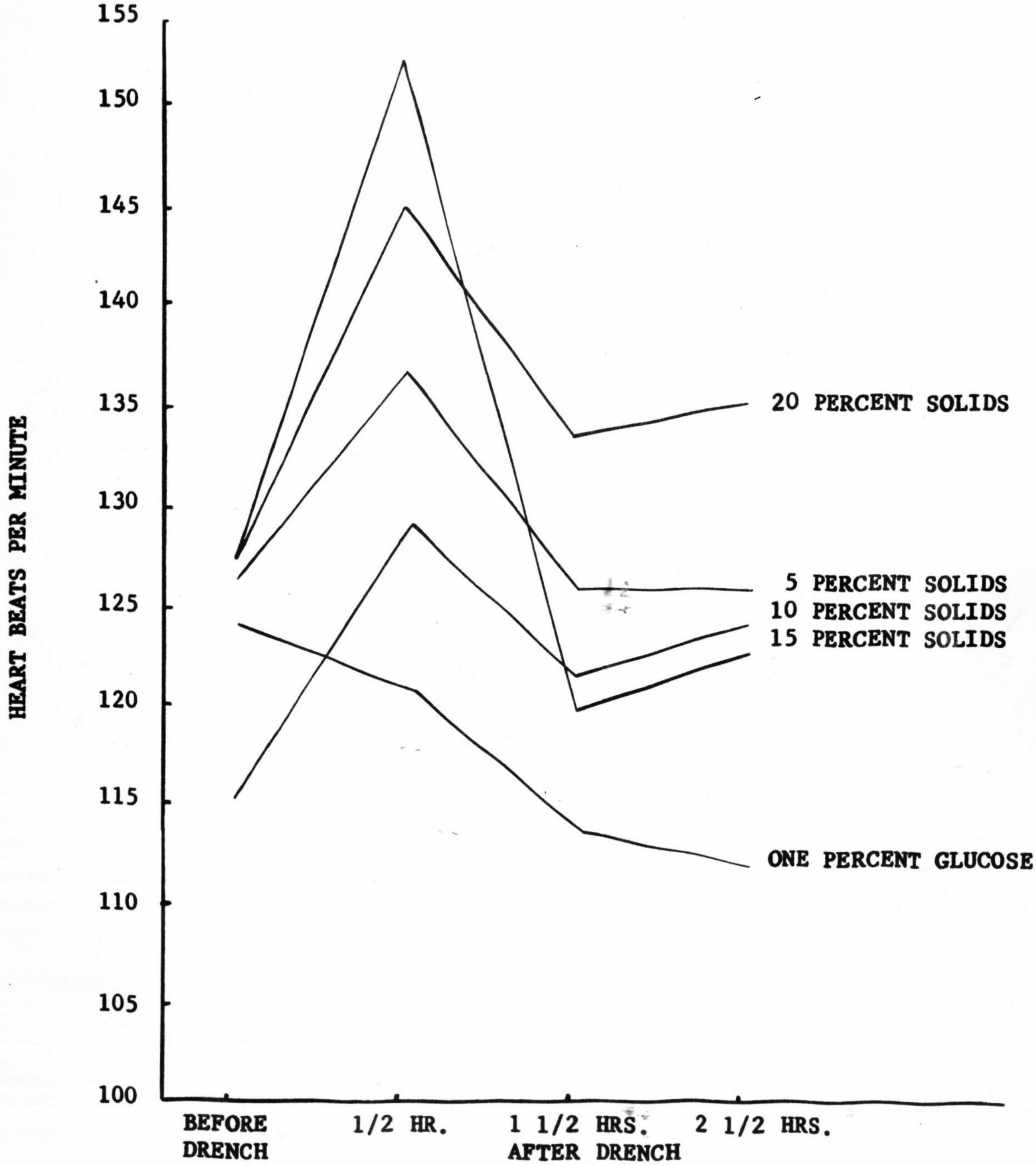

Figure 2. Comparison of Average Heart Rates

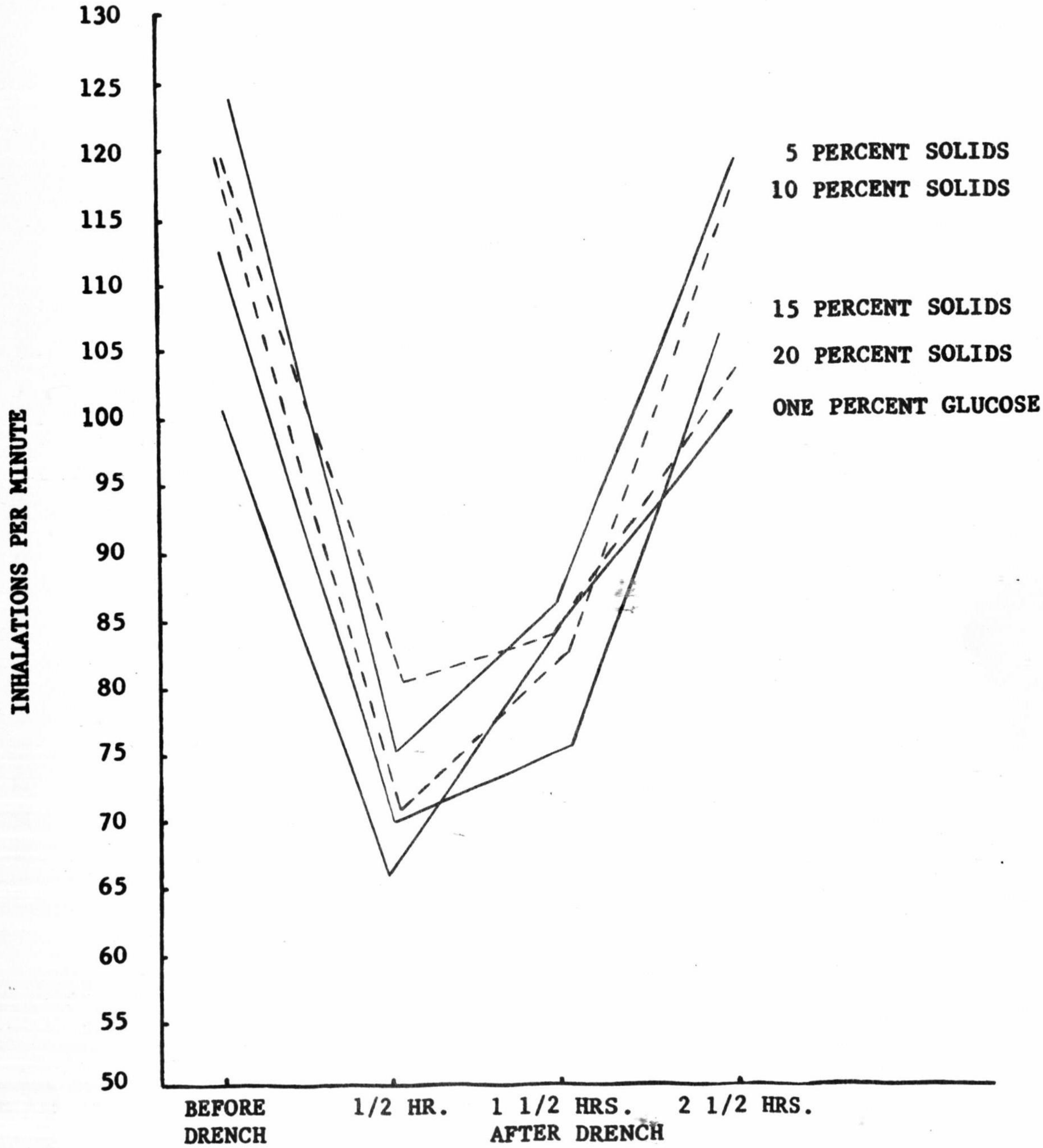

Figure 3. Comparison of Average Respiratory Rates

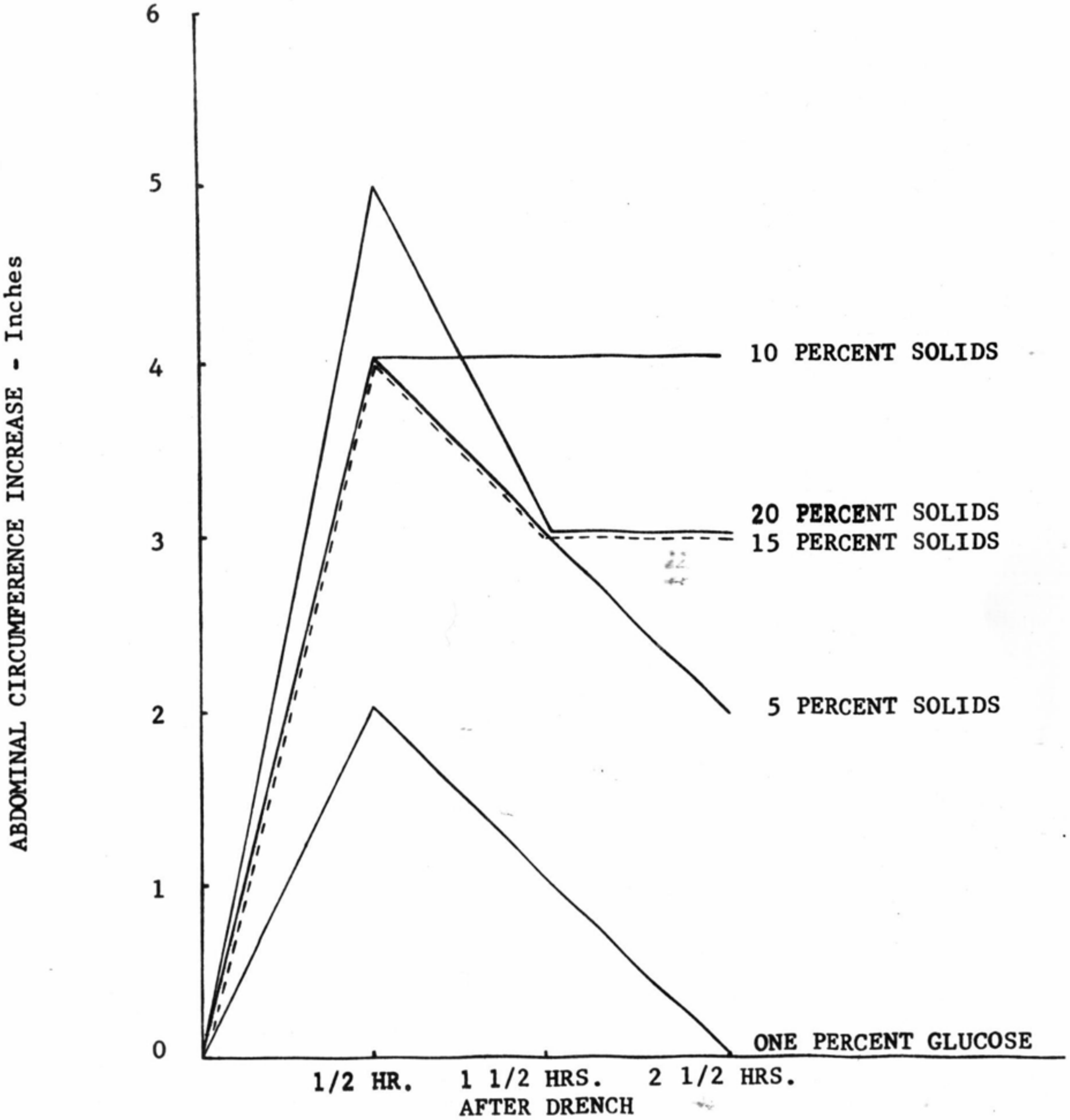

Figure 4. Comparison of Average Abdominal Circumference

within the first hour after drenching and then increased (Fig. 3). The average abdominal circumference reached a maximum the first hour after drenching and then decreased (Fig. 4).

<u>Carotid Arterial Blood Pressure</u>

Calf no. 187, as shown in Table XI, bloated only four times. This animal had one case of slight bloat, two cases of moderate bloat, and then died while seriously bloated on June 24, 1958. There was no definite trend as to the heart rate. The respiratory rate about doubled after pasturing and the blood pressure increased considerably when seriously bloated.

Table XIV shows the sequence of events of calf no. 187 when seriously bloated. The highest recording of blood pressure was 230 mm. Hg., 40 minutes prior to death. Ten minutes before death, the blood pressure was 220 mm. Hg. and no recording was made after that. There was little change in heart and respiratory rates while the animal was seriously bloated.

Calf no. 189 had slight bloat ten times, moderate bloat six times, and serious bloat once (Table XII). The heart rate increased most when the animal was seriously bloated. The same was true of the blood pressure. The respiratory rate, as before, increased following pasturing, but changed very little while the animal bloated.

The events which took place while calf no. 189 was bloated are given in Table XIV. The highest blood pressure recorded was 255 mm. Hg., but it probably went higher since the mercury manometer used would not record any higher. The blood pressure and heart rate decreased whenever the gas was released from the rumen. When the blood pressure was 255 mm. Hg.

TABLE XI. HEART RATE, RESPIRATORY RATE AND SYSTOLIC BLOOD PRESSURE OF CALF NO. 187

	Before Pasturing			After Pasturing			Slight Bloat			Moderate Bloat			Severe Bloat		
Date	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP
6-10-58	120	48	160	136	48	165									
6-11-58	128	48	170	128	48	185									
6-12-58	116	36	185	116	80	175									
6-16-58	112	24	170	124	80	180									
6-17-58	104	52	175	124	96	175									
6-18-58	96	32	190	100	108	170									
6-19-58	88	60	185							120	76	170			
6-20-58	112	36	165				120	48	155						
6-23-58	116	32	180							108	76	200			
6-24-58	84	20	160										124	80	230
Average	108	39	174	121	77	175	120	48	155	114	76	185	124	80	230
Change				+13	+38	+1	+12	+9	-19	+6	+37	+11	+16	+41	+56

HR = Heart rate (min.)
RR = Respiratory rate (min.)
BP = Blood pressure (mm. Hg.)

TABLE XII. HEART RATE, RESPIRATORY RATE AND SYSTOLIC BLOOD PRESSURE OF CALF NO. 189

Date	Before Pasturing			After Pasturing			Slight Bloat			Moderate Bloat			Severe Bloat		
	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP
6-10-58	124	56	155	124	56	175									
6-11-58	88	52	180	128	28	145									
6-12-58	84	32	180	96	60	175									
6-16-58	84	24	155	104	72	175									
6-17-58	84	60	175	104	88	170									
6-18-58	72	28	180				96	52	190						
6-19-58	72	32	180	96	32	185									
6-20-58	84	32	175				96	64	180						
6-23-58	104	52	180	128	56	185									
6-24-58	96	32	170	96	42	180									
6-25-58	72	20	190	96	32	185									
6-26-58	84	24	180	104	48	185									
7-1-58	84	28	180				112	56	215						
7-9-58	64	36	200	84	56	200									
7-15-58	56	28	200	104	52	195									
7-16-58	72	48	190	80	80	195									
7-17-58	64	36	195	108	80	195									
7-18-58	80	40	185	112	88	150									
7-21-58	80	40	170	90	60	195									
7-22-58	80	60	170				108	128	195						
7-23-58	72	36	160				84	68	200						
7-24-58	68	36	160	88	80	200									
7-28-58	68	60	185				96	68	190						
7-29-58	64	48	180				96	60	200						

Table XII. Heart Rate, Respiratory Rate and Systolic Blood Pressure of Calf No. 189 (continued)

	Before Pasturing			After Pasturing			Slight Bloat			Moderate Bloat			Severe Bloat		
Date	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP
7-30-58	64	52	170	96	52	170									
8-15-58	84	32	170										160	60	255
8-19-58	64	32	170				104	80	205						
8-20-58	72	36	195				104	104	220						
8-21-58	64	32	205	96	76	215									
8-22-58	68	52	185				96	76	215						
8-26-58	64	32	165	88	72	190									
8-27-58	72	28	165	84	108	175									
9-9-58	68	48	185							84	72	200			
9-10-58	72	20	195							100	60	210			
9-12-58	68	32	190							92	56	210			
9-16-58	64	20	200							96	84	220			
9-17-58	72	36	175							80	56	205			
9-18-58	64	20	185							72	44	205			
Average	75	37	180	100	63	183	99	76	201	87	62	208	160	60	255
Change				+25	+26	+3	+24	+39	+21	+12	+25	+28	+85	+23	+75

TABLE XIII. HEART RATE, RESPIRATORY RATE AND SYSTOLIC BLOOD PRESSURE OF CALF NO. 193

	Before Pasturing			After Pasturing			Slight Bloat			Moderate Bloat			Severe Bloat		
Date	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP	HR	RR	BP
6-10-58	108	44	165	120	44	145									
6-11-58	96	32	115	120	64	185									
6-12-58	88	32	175	112	68	170									
6-17-58	124	52	175	124	52	195									
6-18-58	88	48	140	100	108	170									
6-19-58	108	60	170	120	28	210									
6-24-58	104	32	180	112	44	185									
7-15-58	80	24	180	104	68	190									
7-16-58	80	20	190	120	84	180									
7-17-58	72	40	185	120	88	185									
7-18-58	72	28	180	96	88	160									
7-21-58	72	24	155	94	112	160									
7-22-58	80	28	160										142	60	240
7-23-58	92	36	180	104	80	175									
7-24-58	80	64	190	96	84	175									
7-28-58	80	40	195				96	92	200						
7-29-58	76	40	180				96	92	185						
7-30-58	72	36	170	96	80	185									
8-22-58	80	32	165				96	76	215						
8-26-58	80	36	180	96	72	190									
8-27-58	68	32	190	104	120	180									
Average	86	37	172	108	76	179	96	87	200				142	60	240
Change				+22	+39	+7	+10	+50	+28				+56	+23	+68

TABLE XIV. SEQUENCE OF EVENTS ON SEVERELY BLOATED ANIMALS

Date	Animal No.	Before Pasturing			Severe Bloat			Time	Remarks
		HR	RR	BP	HR	RR	BP		
6-24-58	187	84	20	160	124	80	230	11:30 A.M.	Very serious; labored breathing; frequent urination and defecation; got up and lay down several times.
					124	80	220	11:40 A.M.	Appeared more quiet, but still in a serious condition.
					120	76	220	11:50 A.M.	Breathing more labored.
					124	76	220	12:00 Noon	Ten minutes after this recording, the animal died.
8-15-58	189	84	32	170	160	60	255	10:50 A.M.	Very serious; labored breathing; frequent urination and defecation. Appeared about ready to fall so used trocar to release free gas.
					112	84	225	10:55 A.M.	Still bloated seriously; breathing not as labored and the animal no longer groaning.
					148	84	255	11:00 A.M.	Becoming more distressed, so tried to relieve more gas through the trocar.
					136	84	240	11:10 A.M.	More gas was let out, and the distress symptoms were less.
					116	72	230	11:20 A.M.	More gas released via the trocar and less distress symptoms.
					108	68	230	11:30 A.M.	Bloated, but no longer serious.
					104	22	230	1:00 P.M.	No longer bloated.
					72	32	220	3:30 P.M.	

Table XIV. Sequence of Events on Severely Bloated Animals (continued)

		Before Pasturing			Severe Bloat				
Date	Animal No.	HR	RR	BP	HR	RR	BP	Time	Remarks
7-15-58	193	80	28	160	142	60	240	10:30 A.M.	Very severe; labored breathing; got up, lay down, groaned; used trocar.
					128	60	200	10:40 A.M.	Distress symptoms not quite as great.
					142	72	230	11:00 A.M.	Became very serious; fell down, so cut rumen open with a knife and let out a frothy mass of ingesta.
					144	108	230	11:07 A.M.	Breathing picked up, and was no longer labored.
					104	96	200	11:15 A.M.	Recovering very well.
					100	88	200	11:20 A.M.	
					100	88	200	11:30 A.M.	
					100	92	185	11:55 A.M.	

and the heart rate 160 beats per minute, the respiratory rate was 60 per minute and extremely labored. However, when gas was released from the rumen, the blood pressure and heart rate decreased; the respiration increased and was less labored. Then as the distress bloating symptoms decreased, the respiration decreased. The blood pressure remained high for several hours.

Four cases of bloat were produced in calf no. 193 (Table XIII). Three of these were slight and the other was severe. Again, the blood pressure (240 mm. Hg.) and heart rate (142) increased considerably when severely bloated and the respiratory rate (60) was labored.

The sequence of events of calf no. 193 while severely bloated are revealed in Table XIV. When gas was released via trocar, the heart rate and blood pressure decreased. Then the animal became extremely distressed again and a rumenotomy was performed. The blood pressure and heart rate remained about the same for a short time and then decreased. The respiratory rate increased and was much freer.

An average heart rate, respiratory rate and blood pressure of calves nos. 187, 189 and 193 are shown in Table XV. The most obvious differences were during severe bloat. The differences in heart rates, respiratory rates and blood pressures during slight and moderate bloat were negligible.

Methemoglobin and Total Hemoglobin

Fifty-three cases of bloat were produced in eighteen cows on alfalfa pasture. Twenty-six of these cases were slight bloat, 19 moderate bloat and 8 severe bloat. Four of the severely bloated animals died, and the other four had to be treated by trocar-cannula or rumenotomy.

TABLE XV. AVERAGE HEART RATES, RESPIRATORY RATES AND SYSTOLIC BLOOD PRESSURES OF CALVES NO. 189, 193 AND 187

	Before Pasturing				After Pasturing				Slight Bloat				Moderate Bloat				Severe Bloat			
Animal	Re-cord-ings	HR	RR	BP	Re-cord-ings	HR	RR	BP	Re-cord-ings	HR	RR	BP	Re-cord-ings	HR	RR	BP	Re-cord-ings	HR	RR	BP
189	38	75	37	180	21	100	63	183	10	99	76	201	6	87	62	208	1	160	60	255
193	21	86	37	172	17	108	76	179	3	96	87	200	-	--	--	---	1	142	60	240
187	10	108	39	174	6	121	77	175	1	120	48	155	2	114	76	185	1	124	80	230
Total	69	269	113	526	44	329	216	537	14	315	211	556	8	201	138	393	3	426	200	725
Average		90	38	175		110	72	179		105	70	185		100	69	196		142	67	242

Table XVI shows the methemoglobin and total hemoglobin values. The average methemoglobin values in grams per 100 ml. of blood are: (normal animals) .539; (slight bloat) .626; (moderate bloat) .457; (severe bloat) .425. There is no great difference between normal and bloated animals. Average total hemoglobin values are as follows: (normal) 10.68; (slight bloat) 10.20; (moderate bloat) 10.51; (severe bloat) 10.67. Again, there was no noticeable difference between normal and bloated animals.

TABLE XVI. METHEMOGLOBIN AND TOTAL HEMOGLOBIN VALUES FOR NORMAL AND BLOATED ANIMALS

		Methemoglobin Values (gm/100 ml. blood)				Total Hemoglobin Values (gm/100 ml. blood)			
Date	Cow No.	Normal	Slight Bloat	Moderate Bloat	Severe Bloat	Normal	Slight Bloat	Moderate Bloat	Severe Bloat
6-12-58	195			.448					
	123		.448						
	86	.448							
	197	.448							
6-13-58	195	.455		.455					
	123	.451		.635					
	86	.632							
	197	.632							
	125		.455						
	185	.455							
6-18-58	123	.740	.931						
	125		.830						
	167		.838						
	86	.762							
6-19-58	187	.538		.733					
	86	.632							
6-20-58	86		.668						
	178		.650						
	189		.650						
	187		.643						
	174	.823							
6-23-58	187			.639					
	86	.668							
6-27-58	123		.740						
	174		.552						
	167	.827							

Table XVI. Methemoglobin and Total Hemoglobin Values for Normal and Bloated Animals (continued)

		Methemoglobin Values (gm/100 ml. blood)				Total Hemoglobin Values (gm/100 ml. blood)			
Date	Cow No.	Normal	Slight Bloat	Moderate Bloat	Severe Bloat	Normal	Slight Bloat	Moderate Bloat	Severe Bloat
6-24-58	187				.545 (died)				
	86	.556							
7-2-58	184	.646	.556						
	125	.523	.440						
	167			.458					
	185		.458						
	123	.639							
7-8-58	86		.552						
	167	.523							
7-11-58	86		.635						
	185	.646	.643						
	123	.635							
7-15-58	188				.722 (died)				
	197	.621							
7-22-58	193				.253				8.433
	189		.253				8.723		
	198	.173							
7-23-58	123			.353					
	86	.455							
7-29-58	123			. 538					
	184	.942	.921						
	86	.838							
8-7-58	184		.740				9.782		
	195		.740				9.782		
	86	.762							

Table XVI. Methemoglobin and Total Hemoglobin Values for Normal and Bloated Animals (continued)

		Methemoglobin Values (gm/100 ml. blood)				Total Hemoglobin Values (gm/100 ml. blood)			
Date	Cow No.	Normal	Slight Bloat	Moderate Bloat	Severe Bloat	Normal	Slight Bloat	Moderate Bloat	Severe Bloat
8-8-58	197	.733	.762			9.483	8.147		
	86	.740							
8-12-58	86	.740				11.412			
	102		.740				11.580		
	184			.736				11.580	
	123		.715				9.626		
8-13-58	123			.448				9.626	
	86	.451				11.076			
8-14-58	123			.354				9.475	
	86	.448							
8-15-58	189	.545			.643	11.412			12.647
	158	.545	.455			11.756	10.744		
	123	.715		.534		10.097		9.782	
8-19-58	123			.625	.444 (died)			10.256	
	158	.455				10.744			
8-21-58	195	.271		.271		10.908		10.908	
	167	.361				12.109			
9-9-58	193				.264 (died)				11.240
	194	.350				8.000			
	189	.354				10.100			
9-10-58	185	.444				12.290			
	195	.177			.220	11.410			10.910
	167		.267				13.190		

Table XVI. Methemoglobin and Total Hemoglobin Values for Normal and Bloated Animals (continued)

		Methemoglobin Values (gm/100 ml. blood)				Total Hemoglobin Values (gm/100 ml. blood)			
Date	Cow No.	Normal	Slight Bloat	Moderate Bloat	Severe Bloat	Normal	Slight Bloat	Moderate Bloat	Severe Bloat
9-16-58	102	.314				11.670			
	189	.350		.267		9.470		11.500	
	195	.224			.311	10.180			10.100
	184	.267		.271		10.420		10.270	
9-17-58	194	.260				9.780			
	195			.401				11.760	
	189			.394				9.390	
	184			.181				11.080	
No. of Cases		(47)	(26)	(19)	(8)	(18)	(8)	(11)	(5)
Average		.539	.626	.457	.425	10.680	10.200	10.510	10.670
Percent of Total Hb.		5.05	6.14	4.35	3.98				

DISCUSSION OF RESULTS

Alfalfa Extract

Saponin is a biochemical constituent of legumes that is believed by several workers to contribute to the causes of bloat. Lindahl *et al.* (63) have experimentally produced bloat by administering saponins isolated from alfalfa. Moore (68), in a preliminary experiment, concentrated the alfalfa saponins along with other solids in alfalfa extract and found an increased severity of bloat as the percent of solids increased. Data presented in this thesis confirm these findings.

Alfalfa juice concentrate containing 20 and 15 percent solids was much more toxic than that containing 10 and 5 percent solids. For example, six animals died from the drenchings with 20 and 15 percent solids while only one died from the 10 and 5 percent solids. Bloat was also more prevalent in the higher concentrations. Almost all bloat was classified as slight. Only once was the rumen distention great enough to be classified as severe bloat, and this was with the 20 percent solids.

The animals which later died were very restless; respiratory pattern was irregular and often labored; eructation appeared to be partially hampered at times; and they were unsteady on their feet. On autopsy, the ruminal ingesta was found to be frothy with some free gas in the rumen.

The respiratory rate in these experiments decreased rapidly after drenching. However, no conclusion could be drawn as to the depressing effect from the concentrate because the respiratory rate of the controls also decreased. This decrease was probably due more to a cooling effect on the animals. It was noted that the respiration was labored in animals that had been sick after these recordings were made. Although this observation was not made on all animals that had died because some animals became sick and died during the night.

The heart rate was generally fastest when the rumen distention was the greatest, and this was the time when the respiratory rate was the slowest. These conditions were similar to those reported by Moore when a detailed series of drenchings on lambs was conducted.

The cause of death in acute bloat appears to be a complex of physiopathological conditions. Impairment of respiration resulting in anoxia, cardiovascular inhibitors, toxins and several other factors, many of which are unknown, are probably all involved. The exact role of saponin in causing death has not been determined, but according to Lindahl, it undoubtedly plays a role in the death of the animal--first, because of its eructation interference, and secondly, by the systemic effects on respiratory cardiovascular functions and central nervous system.

Carotid Arterial Blood Pressure

No report has been made as to the effect on blood pressure of animals suffering from acute legume bloat. Dougherty (32) showed some effects on blood pressure from intraruminal insufflation which might be comparable. Phillipson (80)(cited by Johns) also did considerable work on intraruminal insufflation and has found results similar to those of Dougherty. The results reported herein compare favorably with those reported by Dougherty and Phillipson.

Systolic blood pressure on three animals changed little until the animals were severely bloated, although on the average, the pressure increased as the degree of bloat increased. This was not so in every case because sometimes the blood pressure was higher when there was no bloat as compared to slight or moderate bloat. During severe bloat, there was a definite rise in blood pressure and this pressure fluctuated with the gas released from the rumen. When gas was released by a trocar, the pressure dropped, and as the animal became more distressed, the pressure increased.

Phillipson reported that sudden rises of blood pressure to over 200 mm. Hg. were common in his inflation experiments. Violent fluctuation in blood pressure occurred and unless pressure in the rumen was released, death soon followed, according to Phillipson. Dougherty likewise reported increased intraruminal pressure caused sharp rises in arterial (carotid) and venous (jugular) blood pressures. The arterial pressure dropped abruptly during eructation.

Eructation in this experiment appeared to be partially inhibited during severe bloat because it was only occasionally that the animal eructated. The heart rate had its greatest rise during severe bloat which was to be expected because of the increased strain on the animal causing the work of the heart to increase. The respiratory rate was always lowest before the animals went on pasture, probably because it was cooler at that time and the animals had not expended much energy. The respiratory rate (average of 67) during severe bloat was rather high and it became very labored at times. The respiratory rate would fluctuate frequently.

Methemoglobin and Total Hemoglobin

Methemoglobin is a hemoglobin derivative formed by the oxidation of the ferrous iron of hemoglobin to ferric iron, Dukes (38). Methemoglobin cannot combine with oxygen in the sense that hemoglobin does and therefore it is useless in the transport of oxygen to the cells. Yarns (97) reported that methemoglobin values of bloated animals were significantly lower than for normal animals. The actual physiological significance of depressed methemoglobin is not well understood, however.

In this experiment, there was no obvious difference between normal and bloated animals as to methemoglobin values. Since methemoglobin is sometimes expressed as percent of total hemoglobin, the latter was also determined. The amount of hemoglobin in the blood is influenced by factors like muscular activity, psychic states and disease. As with methemoglobin, there was little difference between normal and bloated animals in total hemoglobin.

SUMMARY AND CONCLUSIONS

1. Alfalfa juice concentrate containing 20 and 15 percent solids was capable of producing a higher incidence of bloat and more toxic effects than that containing 10 and 5 percent solids. Apparently, alfalfa saponins can be used to produce bloat either by producing frothy ruminal contents, inhibiting eructation, or by other complex actions such as respiratory interference. The exact cause of death in the lambs could not be determined.
2. A surgical method for measuring blood pressure has been developed whereby the carotid artery is exteriorized and sutured into a flap of skin. Pressure is then recorded by a sphygmomanometer.
3. Systolic blood pressure increased markedly when the animals became severely bloated. There was little difference in pressure of slight and moderate bloat.
4. Heart rate had its greatest increase when animals were severely bloated. Apparently, there is quite a strain on the heart during severe bloat.
5. Respiratory rate changed little except that during severe bloat it was extremely labored. Therefore, impairment of respiration is likely to occur only during severe bloat.
6. There was no great difference in methemoglobin and total hemoglobin between normal and bloated animals. Consequently, from these data there is little evidence that the bloat syndrome has any effect upon the formation of methemoglobin.

LITERATURE CITED

(1) Alder, Cited by Johns, "Bloat", Vet. Reviews and Annotations, vol. 2, 107-134, Jarrold & Sons, Ltd: Norwich, Great Britain, 1956.

(2) Barrentine, B. F., Shawver, C. B., and Williams, L. W., "Bloat Studies and Observations", (abs.) J. Animal Sci., vol. 13, 1006, Am. Soc. of An. Prod.: Albany, N. Y., 1954.

(3) Best, C. H., and Taylor, N. B., The Physiological Basis for Medical Practice, 5th Ed., Williams and Wilkins Company: Baltimore, Maryland, 1950.

(4) Blake, J. T., "Evaluation of Various Physical and Nutritional Factors Related to Bloat", Ph.D. Thesis, Iowa State College, 1955.

(5) Boda, J. M., "Studies on the Experimental Production and Prevention of Bloat in Cattle. II. The Influence of Dehydration on the Bloat-Producing Ability of Alfalfa", J. Dairy Sci., vol. 41, 295-301, Am. Dairy Sci. Assoc.: Champaign, Ill., 1957.

(6) Boda, J. M., Silver, B. S., Colvin, H. W., Jr., and Cole, H. H., "Studies on the Experimental Production and Prevention of Bloat in Cattle. I. The Influence of the Intrarumen Administration of Fresh Eggwhite on the Production of Bloat", J. Dairy Sci., vol. 40, 759, Am. Dairy Sci. Assoc.: Champaign, Ill., 1957.

(7) Clark, C. H., "The Nerve Control of Rumination and Reticulo-Ruminal Motility", Am. J. Vet. Res., vol. 14, 376-384, Am. Vet. Med. Assoc.: Chicago, Ill., 1953.

(8) Clark, R., "The Use of Anti-Frothing Agents in the Treatment of Acute Bloat", Onderstepoort, J. South African Vet. Med. Assoc., vol. 21, 173-178, Pretoria, S. Africa, 1950.

(9) Clark, R., "The Paralytic Action of Histamine on the Ruminal Muscultine", Onderstepoort, J. South African Vet. Med. Assoc., vol. 31, 13, Pretoria, S. Africa, 1950.

(10) Clark, R., and Lombard, W. A., "Studies on the Alimentary Tract of Merino Sheep in South Africa. XXII. The Effect of the pH of The Ruminal Contents on Ruminal Motility", Onderstepoort, J. Vet. Res., vol. 25, 79-92, Pretoria, S. Africa, 1951.

(11) Clark, R., and Quin, J. I., "Studies on the Alimentary Tract of Merino Sheep in South Africa. XIII. The Role of Prussic Acid in the Aetiology of Acute Bloat", Onderstepoort, J. Vet. Sci. Animal Ind., vol. 20, 209-212, Pretoria, S. Africa, 1945.

Clark, R., and Weiss, K. E., "Factors Contributing Towards Bloat in Ruminants", Onderstepoort, J. South African Vet. Med. Assoc., vol. 23, 103-106, Pretoria, S. Africa, 1952.

Clark, R., and Weiss, K. E., "Reflex Salivation in Sheep and Goats Initiated by Mechanical Stimulation of the Cardiac Area of the Forestomachs", Onderstepoort, J. South African Vet. Med. Assoc., vol. 23, 163, Pretoria, S. Africa, 1952.

Clark, R., Oyaert, W., and Quin, J. I., "Studies on the Alimentary Tract of the Merino Sheep in South Africa. XXI. The Toxicity of Urea to Sheep Under Different Conditions", Onderstepoort, J. Vet. Res., vol. 25, 73-78, Pretoria, S. Africa, 1951.

Cole, H. H., "Chemical and Physical Factors in the Etiology of Acute Bloat on Legume Pasture", Proc. Conference on Rumen Function, Chicago, Ill, 1952.

Cole, H. H., and Kleiber, M., "Studies on Ruminal Gas Formation and on Consumption of Alfalfa Pasture by Cattle", J. Dairy Sci., vol. 31, 1016-1023, Am. Dairy Sci. Assoc.: Champaign, Ill., 1948.

Cole, H. H., Mead, S. W., and Kleiber, M., "Bloat in Cattle", Calif. Agr. Expt. Sta. Bull. 662, 1942.

Cole, H. H., Mead, S. W., and Regan, W. M., "Production and Prevention of Bloat in Cattle on Alfalfa Pasture", J. Animal Sci., vol. 2, 285-294, Am. Soc. An. Prod.: Albany, N. Y., 1943.

Cole, H. H., Huffman, C. F., Kleiber, M., Olson, T. M., and Schalk, A. F., "A Review of Bloat in Ruminants", J. Animal Sci., vol. 4, 183-236, Am. Soc. An. Prod.: Albany, N. Y., 1945.

Cole, H. H., Dougherty, R. W., Huffman, C. F., Hungate, R. E., Kleiber, M., and Maclay, W. D., "A Review of Bloat in Ruminants", Nat. Acad. Sci. - Nat. Res. Council, Publ. 388, Washington, D. C., 1955.

Cooper, H. P., "Relations of Phosphorous Deficiency to Acute Bloat in Ruminants", The Southeastern Vet., vol. 8, 120-125, Publisher Unknown, 1957.

Dain, J. A., Neal, A. L., and Dougherty, R. W., "The Occurrence of Histamine and Tyramine in Rumen Ingesta of Experimentally Over-Fed Sheep", J. Animal Sci., vol. 14, 930-935, Am. Soc. An. Prod.: Albany, N. Y., 1955.

Dougherty, R. W., "Physiological Studies of Induced and Natural Bloat in Dairy Cattle", J. Am. Vet. Med. Assoc., vol. 96, 43-46, Am. Vet. Med. Assoc.: Chicago, Ill., 1940.

(24) Dougherty, R. W., "Further Investigations of Rumen Gases and Bloat in Ruminants", J. Am. Vet. Med. Assoc., vol. 99, 110-114, Am. Vet. Med. Assoc.: Chicago, Ill., 1941.

(25) Dougherty, R. W., "A Study of Drugs Affecting the Motility of the Bovine Rumen", Cornell Vet., vol. 32, 269-280, Cornell Vet., Inc., James Law Hill: Ithaca, N. Y., 1942.

(26) Dougherty, R. W., "Analysis of Gas and Ingesta of Bloated Cattle", Am. J. Vet. Res., vol. 3, 401, Am. Vet. Med. Assoc.: Chicago, Ill., 1942.

(27) Dougherty, R. W., "The Continuing Quest for the Cause of Bloat in Ruminants", J. Am. Vet. Med. Assoc., vol. 122, 914, Am. Vet. Med. Assoc.: Chicago, Ill., 1953.

(28) Dougherty, R. W., and Cello, R. M., "Study of Toxic Factors in the Rumen Ingesta of Cows and Sheep", Cornell Vet., vol. 39, 403-413, Cornell Vet., Inc., James Law Hill: Ithaca, N. Y., 1949.

(29) Dougherty, R. W., and Cello, R. M., "Study of Toxic Factors in Rumen Ingesta of Cows and Sheep", Am. Vet. Med. Assoc., "Proceedings Book", pp. 130-137, Eighty-ninth Annual Meeting: Atlantic City, 1952.

(30) Dougherty, R. W., and Habel, R. E., "The Cranial Esophageal Sphincter: It's Action and It's Relation to Eructation in Sheep as Determined by Cinefluorography", Cornell Vet., vol. 45, 459-464, Cornell Vet., Inc., James Law Hill: Ithaca, N. Y., 1955.

(31) Dougherty, R. W., and Meredith, C. D., "Cinefluorographic Studies of the Ruminant Stomach and of Eructation", Am. J. Vet. Res., vol. 16, 96-100, Am. Vet. Med. Assoc.: Chicago, Ill., 1955.

(32) Dougherty, R. W., Meredith, C. D., and Barrett, R. B., "Physiological Effects of Insufflation of the Stomach of Sheep", Am. J. Vet. Res., vol. 16, 79-90, Am. Vet. Med. Assoc.: Chicago, Ill., 1955.

(33) Dougherty, R. W., Habel, R. E., and Bond, H. E., "Esophageal Innervation and the Eructation Reflex in Sheep", Am. J. Vet. Res., vol. 19, 115-128, Am. Vet. Med. Assoc., 1958.

(34) Dracy, A. E., "The Effect of Atropine on the Gas Accumulation of the Normal Ruminant", Proc. S. D. Acad. Sci., vol. 34, 121-123, Univ. S. D.: Vermillion, S. D., 1955.

(35) Dracy, A. E., and Jordan, R. M., Unpublished data.

(36) Dracy, A. E., and Moore, C. L., Unpublished data.

(37) Dracy, A. E., Jordan, R. M., and Shaffhansen, D. D., "The Effect of Clamping the Esophagus upon Gas Accumulation in the Rumen and the Well-being of Sheep", Proc. S. D. Acad. Sci., vol. 33, 175-177, Univ. S. D.: Vermillion, S. D., 1954.

(38) Dukes, H. H., "The Physiology of Domestic Animals", 7th Ed., Comstock Publishing Company, Inc.: Ithaca, N. Y., 1955.

(39) Dzink, H. E., and Sellers, A. F., "Physiological Studies of the Vagal Nerve Supply to the Bovine Stomach. II. Studies on the Eructation Mechanism in Adult Cattle", Am. J. Vet. Res., vol. 16, 499-504, Am. Vet. Med. Assoc.: Chicago, Ill., 1955.

(40) Ellenberger, W., Cited by Weiss, "Physiological Studies on Eructation in Ruminants", Onderstepoort, J. Vet. Res., vol. 26, 251-283, Pretoria, S. Africa, 1953.

(41) Evans, W. C., and Evans, E. T. R., "Relation of a Clover Juice Factor Causing Paralysis of Smooth Muscle to Bloat in Ruminants", Nature, vol. 163, 173-377, St. Martin's Press, Inc.: N. Y., 1949.

(42) Evelyn, K. A., and Malloy, H. T., "Microdetermination of Oxyhemoglobin, Methemoglobin and Sulfhemoglobin in a Single Sample of Blood", J. B. C., vol. 126, 655-662, Am. Soc. Biol. Chem., Inc.: Baltimore, Md., 1938.

(43) Ferguson, T. H., and Ferguson, S. E., "Acute Bloating in Cattle", North Am. Vet., vol. 15, 27-29, Am. Vet. Publ., Inc.: Evanston, Ill., 1934.

(44) Ferguson, W. S., "A Possible Relation Between the Action of Clover Juice on Isolated Muscle and 'Bloat' in Cattle", Nature, vol. 161, 816-817, St. Martin's Press, Inc.: N. Y., N. Y., 1948.

(45) Ferguson, W. S. and Terry, R. A., "Bloat Investigations", J. Agr. Sci., vol. 46, 257-266, Cambridge Univ. Press: N. Y., N. Y., 1955.

(46) Ferguson, W. S., Ashworth, D. B., and Terry, R. A., "Nature of Muscle-Inhibiting Compound in Lucerne and Its Possible Connection with Bloat in Cattle", Nature, vol. 163, 606, St. Martin's Press, Inc.: N. Y., 1949.

(47) Ferguson, W. S., Ashworth, D. B., and Terry, R. A., "Identity of Muscle-Inhibiting Flavone in Lucerne", Nature, vol. 166, 116, St. Martin's Press, Inc.: N. Y., 1950.

(48) Graham, W. R., Turner, C. W., Gomex, E. T., "A Method for Obtaining Arterial Blood from the Goat", Mo. Agr. Exp. Sta. Res. Bull. 260, 1937.

(49) Hale, W. H., and King, R. P. Cited by Cole, _et al._, "A Review of Bloat in Ruminants", _Nat. Acad. Sci. - Nat. Res. Council_, Pub. 388, Washington, D. C., 1955.

(50) Harris, H. E., McDaniel, A. H., and Roark, C. B., "Cattle Bloat on Clover Pastures", _La. State Univ. Agr. Exp. Sta. Bull. 469_, 1952.

(51) Heath, D. F., and Park, P. O., "An Irreversible Cholinesterase Inhibitor in White Clover", _Nature_, vol. 172, 206, St. Martin's Press, Inc.: N. Y., 1953.

(52) Hungate, R. E., Dougherty, R. W., Bryant, M. P., and Cello, R. M., "Microbiological and Physiological Changes Associated with Acute Indigestion in Sheep", _Cornell Vet._, vol. 42, 423-449, Cornell Vet., Inc., James Law Hill: Ithaca, N. Y., 1952.

(53) Iggo, A., "Tension Receptors in the Stomach and the Urinary Bladder", _J. Physiol._, vol. 123, 593-607, Cambridge Univ. Press: N. Y., N. Y., 1955.

(54) Jacobson, D. R., and Lindahl, T. L., "Ingesta Volume Increase in Rumen Contents of Cattle on Normal and Bloat-Producing Rations", (abs.), _J. Dairy Sci._, vol. 38, 606, Am. Dairy Sci. Assoc.: Champaign, Ill., 1955.

(55) Jacobson, D. R., and Lindahl, T. L., "Studies on Biochemical, Physical and Bacteriological Factors Involved in Feedlot Bloat", _Univ. Md. Misc. Pub. 238_, vol. 9, 1955.

(56) Jacobson, D. R., Lindahl, T. L., McNeill, J. J., Shaw, J. C., Doetsch, R. N., and Davis, R. E., "Feedlot Bloat Studies. II. Physical Factors Involved in the Etiology of Frothy Bloat", _J. Animal Sci._, vol. 16, 515-524, Am. Soc. An. Prod.: Albany, N. Y., 1957.

(57) Jacobson, N. L., Espe, D., and Cannon, C. Y., "Factors Modifying the Rate of Fermentation of Ingesta and Their Possible Relation to Bloat in Dairy Cattle", _J. Dairy Sci._, vol. 25, 785-799, Am. Dairy Sci. Assoc.: Champaign, Ill., 1942.

(58) Johns, A. T., "Bloat in Cattle on Red Clover", I. _New Zealand J. Sci. Tech._, vol. 36, 289-320, Dept. Scientific and Industrial Res.: Wellington, New Zealand, 1954.

(59) Johns, A. T., "Bloat in Cattle on Red Clover", II. _New Zealand J. Sci. Tech._, vol. 37, 323, Dept. Scientific and Industrial Research: Wellington, New Zealand, 1955.

(60) Johns, A. T., "Bloat", _Vet. Reviews and Annotations_, vol. 2, 107-134, Jarrold & Sons, Ltd.: Norwich, Great Britain, 1956.

(61) Lindahl, I. L., and Davis, R. E., "Some Factors in Feedlot Bloat", (abs.) J. Animal Sci., vol. 13, 1024, Am. Soc. An. Prod.: Albany, N. Y., 1954.

(62) Lindahl, I. L., Cook, A. C., Davis, R. E., and Maclay, W. E., "Preliminary Investigations on the Role of Alfalfa Saponin in Ruminant Bloat", Science, vol. 119, 157-158, Am. Assoc. Advanc. Sci.: Washington, D. C., 1954.

(63) Lindahl, I. L., Davis, R. E., Tertell, R. T., Whitmore, G. E., Shalkep, W. T., Dougherty, R. W., Thompson, C. R., Van Otta, G. R., Bickoff, E. M., Walter, E. D., Livingston, A. G., Gugglz, J., Wilson, R. H., Sideman, M. B., and DeEds, F., "Alfalfa Saponins Studies of Their Chemical Pharmacological and Physiological Properties in Relation to Ruminant Bloat", U. S. Dept. Agr. Tech. Bull. No. 1161, U.S.D.A., Washington, D. C., 1957.

(64) Mangold, E., and Klein, W., Cited by Blake, "Evaluation of Various Physical and Nutritional Factors Related to Bloat", Ph.D. Thesis, Iowa State College, 1955.

(65) Marshall, S. P., Arnold, D. P. T., and Becker, R. B., "Postpartum Development of the Bovine Stomach Compartments and Observation on Some Characteristics of Their Contents", (abs.) J. Dairy Sci., vol. 33, 379, Am. Dairy Sci. Assoc.: Champaign, Ill., 1950.

(66) McCandlish, A. C., Cited by Cole, et al., "A Review of Bloat in Ruminants", J. Animal Sci., vol. 4, 183-236, Am. Soc. An. Prod.: Albany, N. Y., 1945.

(67) Mead, S. W., Cole, H. H., and Regan, W. M., "Further Studies on Bloat", J. Dairy Sci., vol. 27, 779-791, Am. Dairy Sci. Assoc.: Champaign, Ill., 1944.

(68) Moore, C. L., "Bloat. Results from Various Drenchings Including Effectiveness of Penicillin for Prevention", M.S. Thesis, South Dakota State College, 1957.

(69) Moore, C. L., Hall, V. A., and Dracy, A. E., "Recent Studies on Bloat", S. D. Farm and Home Res., vol. 8, 21-23, Dairy Dept.: South Dakota State College, 1957.

(70) Moore, C. L., Hall, V. A., and Dracy, A. E., "Bloat. Results from Various Drenchings Including Effectiveness of Penicillin for Prevention", (abs.) J. Dairy Sci., vol. 40, 616, Am. Dairy Sci. Assoc.: Champaign, Ill., 1957.

(71) Nichols, R. E., "A Note on Eructation in Sheep as Related to Rumen Fluid Level and Gas Insufflation", Am. J. Vet. Res., vol. 12, 199-200, Am. Vet. Med. Assoc.: Chicago, Ill., 1951.

(72) Nichols, R. E., "Why do Cows and Sheep Bloat?", Vet. Med., vol. 49, 91-95, Vet. Med. Pub. Co.: Kansas City, Mo., 1954.

(73) Olson, T. M., "Bloat in Dairy Cattle", S. D. Agr. Exp. Sta. Circ. 27, 1-8, 1940.

(74) Olson, T. M., "Ruminal Gases in Normal and Bloated Animals", (abs.) J. Dairy Sci., vol. 25, 684-685, Am. Dairy Sci. Assoc.: Champaign, Ill., 1942.

(75) Olson, T. M., "Bloat in Dairy Cattle", S. D. Agr. Exp. Sta. Circ. 52, 1-11, 1944.

(76) Oyaert, W., Quin, J. I., and Clark, R., "Studies on the Alimentary Tract of the Merino Sheep in South Africa. XIX. The Influence of Sulphanilamide on the Activity of the Ruminal Flora of Sheep and Cattle", Onderstepoort, J. Vet. Res., vol. 25, 59-65, Pretoria, S. Africa, 1951.

(77) Parsons, A. R., Hall, G., and Thomas, W. E., "Forage Extracts Which Cause Bloat or Death When Administered Orally to Sheep", (abs.) J. Animal Sci., vol. 11, 772-773, Am. Soc. An. Prod.: Albany, N. Y., 1952.

(78) Parsons, A. R., Newmann, A. L., Whitehair, C. K., and Sampson, J., "Isolated Gut and Rumen Motility as Affected by Extracts from Bloat Producing Forages", J. Animal Sci., vol. 74, 403-411, Am. Soc. An. Prod.: Albany, N. Y., 1955.

(79) Parsons, A. R., Whitehair, C. K., and Newmann, A. L., "Bloat and Rumen Motility as Affected by Forage Extracts and Other Related Substances", (abs.) J. Animal Sci., vol. 12, 926-927, Am. Soc. An. Prod.: Albany, N. Y., 1953.

(80) Phillipson, A. T., Cited by Johns, A. T., "Recent Developments in Bloat Research", Vet. Review and Annotations, vol. 4, 17-31, Jarrold & Sons, Ltd.: Norwich, Great Britain, 1958.

(81) Quin, J. I., "Studies on the Alimentary Tract of Merino Sheep in South Africa. VII. Fermentation of the Forestomach of Sheep", Onderstepoort, J. Vet. Sci. Animal Ind., vol. 18, 91-112, Pretoria, S. Africa, 1943.

(82) Quin, J. I., "Studies on the Alimentary Tract of Merino Sheep in South Africa. VIII. The Pathogenesis of Acute Tympanites (Bloat)", Onderstepoort, J. Vet. Sci. Animal Ind., vol. 18, 113-118, Pretoria, S. Africa, 1943.

(83) Quin, J. I., and Van Der Wath, J. G., "Studies on the Alimentary Tract of Merino Sheep in South Africa. V. The Motility of the Rumen Under Various Conditions", Onderstepoort, J. Vet. Sci. Animal Ind., vol. 11, 361, Pretoria, S. Africa, 1938.

(84) Schalk, A. F., "We're Finding Answers to the Bloat Problem", Successful Farming, vol. 43, 66, Meredith Pub. Co.: Des Moines, Ia., 1945.

(85) Schalk, A. F., and Amadon, R. S., "Physiology of the Ruminant Stomach (Bovine) Study of the Dynamic Factors", N. D. Agr. Exp. Sta. Bull. 216, 1928.

(86) Schambye, P., "A New Technique for Exteriorizing the Carotid Artery", Nord. Vet. Med., vol. 3, 1015-1018, Copenhagen, Den., 1951.

(87) Shaw, R. A., and Jackson, H. D., "Toxic Concentrates from Ladino Clover", J. Animal Sci., vol. 16, 3, Am. Soc. An. Prod.: Albany, N. Y., 1957.

(88) Smith, C. K., and Huffman, C. F., "Frothy Bloat", Proc. Conf. on Rumen Functions, Chicago, p. 7, 1953.

(89) Smith, C. K., Brunner, J. R., Huffman, C. F., and Duncan, C. W., "Experimental Production of Frothy Bloat in Cattle", (abs.) J. Animal Sci., vol. 12, 932, Am. Soc. An. Prod.: Albany, N. Y., 1953.

(90) Stevens, C. E., and Sellers, A. F., Proc. Conf. on Rumen Function, Chicago, p. 28, 1958.

(91) Thomas, E. E., Proc. Conf. on Rumen Functions, Chicago, p. 3, 1951.

(92) Wallace, V. P., (Personal Communication), South Dakota State College, 1958.

(93) Weiss, K. E., "Physiological Studies on Eructation in Ruminants", Onderstepoort, J. Vet. Res., vol. 26, 251-283, The Government Printer, Pretoria, 1953.

(94) Weiss, K. E., "The Significance of Reflex Salivation in Relation to Froth Formation and Acute Bloat in Ruminants", Onderstepoort, J. Vet. Res., vol. 26, 241-250, Pretoria, S. Africa, 1953.

(95) Wester, J., Cited by Cole, et al., "A Review of Bloat in Ruminants", Nat. Acad. Sci. Nat. Res. Council Publ. 388, Washington, D. C., 1955.

(96) Williams, E. I., Cited by Johns, "Bloat", Vet. Reviews and Annotations, vol. 2, 107-134, Jarrold & Sons Ltd.: Norwich, Great Britain, 1956.

(97) Yarns, D. A., "The Blood Picture in Correlation with Bloat", M.S. Thesis, South Dakota State College, 1958.